MAMMOGRAPHIC BREAST DENSITY AS A MEDIATOR AND SURROGATE MARKER FOR BREAST CANCER RISK

BY

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DISSERTATION

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ABSTRACT

Women with the highest mammographic density have a four to six-fold increased risk of breast cancer when compared to the ones with the least dense breasts. Mammographic breast density has also been associated with a wide array of factors related to the risk of breast cancer including age, menopausal status, age at first live birth, parity, body mass index, physical activity, alcohol consumption, hormone replacement therapy, endogenous levels of IGF-I and prolactin, family history of breast cancer, tamoxifen use and others. A question of interest is whether mammographic density is in the pathway by which these factors are related to breast cancer. To address this question, we conducted causal mediation analyses on two datasets using a newly developed statistical approach based on the counterfactual framework to examine the extent to which mammographic density acts as a mediator. The first dataset is pooled from four case-control studies performed in the western Washington state, contains 547 breast cancer cases (ascertained from a local Surveillance, Epidemiology, and End Results Program registry) and 472 controls (ascertained by random digit dialing) who had screening mammograms under age 50. The second dataset is from the Mayo Mammography Health Study (MMHS), which is a prospective cohort, comprised of 19,924 women (51.2% adjusted response rate) ages 35 and over, residing in the tri-state region (Minnesota, Iowa, and Wisconsin) surrounding the Mayo Clinic in Rochester, MN, without a history of breast cancer, who were scheduled for a screening mammogram at the Mayo Clinic between October 2003 and September 2006. Previous analyses from these two datasets have shown associations between some breast cancer risk factors and mammographic density. Results showed that mammographic density partially mediated the associations for some breast cancer risk factors such as breast calcifications, being parous, history of breast biopsy/aspiration/lumpectomy, and current use of hormone replacement therapy (HRT), but not factors such as a first-degree

family history of breast cancer and age at first live birth, history of smoking, age at menopause. These results help us better understand the pathways and mechanisms whereby a risk factor may cause breast cancer. It also helps inform and refine clinical and public health interventions for breast cancer by assessing the relative importance of different pathways.

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CHAPTER 1: INTRODUCTION

High breast density is a strong and well-established risk factor for breast cancer [1, 2]. Currently, the best way to detect breast density is through a mammogram. Fatty tissue in the breast is relatively transparent to x-rays and appears dark on mammograms. Fibroglandular tissue, which consists of epithelial cells that line the ducts and their supporting fibrous connective tissue, is more radiologically dense and appears light on mammograms. The proportion of a mammogram that is dense is thus an indirect measure of the amount of epithelial tissue in the breast. Since, biologically, this is the tissue from which mammary carcinomas arise, the greater the percent mammographic density, the greater the number of cells available for malignant transformation. Percent dense volume is the proportion of fibroglandular (dense) tissue in the breast. Percent mammographic density (PMD), or the proportion of the breast with densities in the breast, is the ratio of the dense area to the total breast area that sums the dense and nondense area. Women with the densest mammographic patterns were estimated to have a four to six-fold increased risk of breast cancer when compared to women with the least dense ones [3]. PMD has been suggested to be a stronger risk factor than absolute dense area [4, 5], which indicates that the ratio between the two tissues is important, or that the nondense area, which is part of the denominator of percent density, is a protective factor for breast cancer risk. Although dense breast tissue may mask tumors on a mammogram, such a strong positive relationship between breast density and risk of breast cancer is regarded as causal rather than correlational [6], which has been consistently observed among studies after controlling for various confounding factors.

Mammographic breast density has been shown to be associated with a wide array of risk factors for breast cancer [1]. Higher density was found in women who were premenopausal, nulliparous, of low BMI (body mass index), low WHR (waist to hip ratio), greater height, had a late age at first birth, younger age, high alcohol consumption, taking combination postmenopausal hormone or with a family history of breast cancer [7-9]. Breast density tends to decrease with older age. Smoking and education were inversely associated with percent density among premenopausal but not postmenopausal women [8]. An inverse association between tissue-based assessment of lobular involution and breast density was also reported [10]. Although, without replication by other studies, some studies observed an association of dietary intake (isoflavones, fat, vitamins, etc.) and

physical activity with breast density [11]. Mammographic breast density can be changed by several exposures that are also known to influence breast cancer risk [9]. For example, tamoxifen, an anti-estrogen, was reported to reduce mammographic breast density as well as the risk of breast cancer [12-15]. Current users of postmenopausal hormone replacement therapy (HRT), especially combined formulations, were found to have higher percent mammographic density and breast cancer risk [16].

These observations suggest that breast density is not only an independent risk factor for breast cancer; it may also be on the causal pathway for many of the established breast cancer risk factors. That is, mammographic breast density has the potential to act as a mediating variable or mediator for breast cancer risk. A mediator differs from confounder in the direction of causality: while mediators lie on the causal pathway between exposure and outcome, confounders influence both the exposure of interest and the outcome [17, 18]. Thus, a mediator occurs temporally after the exposure — it is both caused by the exposure variable and is a cause of the outcome.

Mediation can exist in both nonrandomized and randomized studies. In randomized controlled trials, the treatments/interventions might influence breast cancer risk through their effects on the intermediate marker, mammographic density. In this case, mammographic density as a mediator can be used as a potential surrogate endpoint. According to the National Institutes of Health (NIH), a surrogate endpoint is "a biomarker intended to substitute for a clinical endpoint" that is undesired [19]. Therefore, when the effect of an intervention on the mediator predicts the effect on the clinical outcome, the mediator can be used as a surrogate endpoint. A surrogate marker is used when the primary clinical endpoint is undesired (e.g., death), or when the number of events is small. Clinical trials to assess the effects of interventions on cancer risk often need to be large and prolonged, and as a result, are expensive. For example, the occurrence of breast cancer is a relatively infrequent event. When breast cancer is the primary endpoint, it is hard to measure and it could take a long time to occur. So it is impractical to conduct a clinical trial to gather a statistically significant number of breast cancer case endpoints in a short time. If mammographic density can serve as a surrogate marker for breast cancer as a measure of the effect of a specific treatment that affects the real clinical endpoint, breast cancer, then clinical trials of breast cancer

prevention could be smaller, shorter, and more cost-effective by just focusing on altering breast density.

A mediator is an intermediate marker that helps explain how or why an independent factor influences an outcome. Several requirements, which were first laid out by Baron and Kenny [20], must be met for a variable to be considered as a mediator: i) the exposure variable should be associated with the mediator, (ii) in the model for the outcome that includes the exposure and mediator, the mediator should be associated with the outcome, (iii) in the model for the outcome that includes only the exposure, the exposure should be associated with the outcome, and (iv) when controlling for the mediator, the association between the exposure and outcome should be reduced, with the strongest demonstration of mediation occurring when the path from the exposure to the outcome variable, when controlling for the mediator, is zero. While requirements (iii) and (iv) have been criticized and challenged by many scholars, the first two requirements have generally been accepted as important for establishing a true mediation relationship. In the context of breast density and breast cancer, these two requirements are: the exposure should be associated with breast density and breast density should predict the risk of breast cancer.

When the exposure is a treatment or intervention in a randomized controlled trial, these two requirements for mediation are also two of the three criteria that need to be met for a marker accepted as a suitable surrogate (substitute for breast cancer), proposed by Prentice [21], and further elaborated by Schatzkin and Gail [22], Freedman, Graubard, and Schatzkin [23], and others. Generally speaking, most good surrogates are expected to be on the pathway from the treatment to the outcome[24]. This means that most good surrogates come from mediators and they need to satisfy the requirements as a mediator. For PMD to be a mediator or a surrogate marker, an exposure or treatment should be associated with PMD and PMD should predict the risk of breast cancer. Boyd and colleagues [25] pointed out the third criterion that if PMD is to serve as a surrogate for breast cancer prevention in intervention trials, then most of the effect of such interventions on breast cancer risk should be mediated by PMD. This means that PMD must be a strong mediator in order to qualify for a good surrogate endpoint for breast cancer. are conceptually distinct, they share considerable statistical similarities.

Mediation analysis can formally assess whether a hypothesized factor mediates the effect of an exposure on an outcome. Mediation analyses are often employed to explore the hypothetical causal mechanisms by which a predictor affects an outcome through a mediator variable. In a mediation analysis, a mediator can be identified by decomposing the total effect of an exposure or treatment on an outcome into two components: an indirect effect operating through a mediator of interest and a direct effect operating through alternative pathways that are independent of the mediator. Traditional mediation analysis often applies the structural equation modeling (SEM) approach, first proposed by Baron and Kenny [20], to estimate the direct and indirect effects. SEM is a general multivariate technique widely used in the social sciences. It uses a conceptual model, path diagram, and a system of linked structural regression-style equations to capture complex and dynamic relationships within a network of observed and unobserved (latent) variables. SEM is fundamentally different from a regression-based approach to mediation. In a regression model, there exists a clear distinction between dependent and independent variables. However, these variables in SEM are a relative concept because a dependent variable in one model equation can become an independent variable in other components of the SEM system. SEM provides a more flexible modeling and attractive graphical modeling interface, and can easily extend to handle multi-level data, repeated measures data, and incomplete data. It was considered superior when there are latent variables and moderated mediation. However, SEMs tend to estimate more types of effects at the price of making additional assumptions. Many of these assumptions have often been ignored so they should be used principally for the purpose of exploratory analysis and hypothesis generation when a broad range of effects are of interest. Furthermore, SEMs generally make assumptions of linearity and normality.

The most common regression-based approach for mediation analysis is to use the traditional “difference method” to estimate the “proportion mediated (PM)” as a measure of the mediated or indirect effect. This is based on the change in the coefficients of exposure on the risk of breast cancer from two regression models with and without adjustment for the mediator. This PM measure is equivalent to the “proportion explained (PE)” index, which was proposed by Freedman et al. [23] and further described by others [26, 27]. Similarly, the PE index can be estimated from the difference in the coefficients of treatment from two regression models with or without adjusting for the mediator.

However, structural equation models and the traditional "difference method" are often criticized for neither adequately accounting for the strong assumptions that need to be met nor addressing issues of confounding in inferring causal relationships [28-31]. The two regression models may not be valid simultaneously and the PM or PE index estimate can often lie outside the probability interval between 0 and 1. There are a few situations when using the "difference method" may lead to biased estimators of indirect effects and thus incorrect conclusions regarding mediation, especially for logistic regressions. These include when there is confounding, when a binary outcome is not rare, or when there is exposure-mediator interaction [24].

In the last few years, new approaches for mediation analysis have been proposed to address some of these limitations by using the counterfactual framework [29, 32-36]. The counterfactual approach emphasizes the identifiability assumptions and conceptual definitions of causal effects, which allows for the decomposition of a total effect into direct and indirect effects, even in models with interactions and nonlinearities. The mediation analysis based on the counterfactual framework specifies a model for the outcome and a model for the mediator and then combining the results of these models to obtain direct and indirect effects. A new approach has recently been developed to statistically assess mediation for a case-control study with a rare outcome [36, 37]. With some modifications, this method can also apply to other study designs. It allows us to identify and separate out the direct and indirect mechanisms of breast cancer development that are acting through or not through mammographic density. A recent study compared the traditional "difference method" and the natural indirect effect (NIE) based on the counterfactual framework and concluded that the "difference method" is always conservative for binary outcomes [38]. This suggests that the "difference method" could only be used to provide evidence for the presence of mediation but not for the absence of mediation.

Increasing evidence suggests that mammographic breast density may be a potential mediator for breast cancer risk. Therefore, it is of interest to find out whether the effects of various risk factors on breast cancer are mediated by breast density. If true, then how much does breast density mediate the effects of these risk factors? Currently, very few studies have attempted to address these questions. The potential mediation role of mammographic density for breast cancer

has not yet been thoroughly examined. It is still uncertain whether any of the risk factors influence breast cancer risk through their associations with mammographic density. In this project, we aim to address these questions and to assess the extent to which the observed association between various known risk factors and breast cancer risk is mediated through mammographic density. It is important to utilize newly developed statistical methods to examine what role mammographic density may play as an intermediate marker, if any, for the association between various risk factors and breast cancer incidence.

The results of this work would provide insights into the pathways and mechanisms involved in the etiology of breast cancer. It may help inform and refine clinical and public health interventions for breast cancer by assessing the relative importance of different pathways. This also adds to the current knowledge of how various factors affect breast cancer risk by applying new statistical methods in order to quantify mediating effects through mammographic density.

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CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

Mammographic breast density is a strong independent risk factor for breast cancer [1-4]. Breast density can be influenced by several risk factors that are known to predict breast cancer risk [1]. These observations have led to the hypothesis that breast density may be on the causal pathway for breast cancer for some of the known risk factors for breast cancer. In recent years, there has been an increasing interest in the potential of percent mammographic density (PMD) serving as a mediator or intermediate biomarker for breast cancer risk. Understanding the role of PMD in the pathway linking these risk factors (i.e., exposures) to breast cancer may help inform breast cancer screening and other prevention practices. It is essential to understand the mechanisms underlying the etiology of breast cancer and thus to effective prevention strategies.

In order for mammographic density to be a mediator, an exposure should be associated with mammographic density and mammographic density should predict the risk of breast cancer [5]. While breast density is a well-established risk factor for breast cancer, not all breast cancer risk factors were found to be related to mammographic density. For example, Raloxifene, a drug that reduces the risk of breast cancer in postmenopausal women, appeared to neither increase nor decrease mammographic density [6]. This suggests that Raloxifene may affect breast cancer risk through alternative pathways that are independent of mammographic density. For some risk factors, there is no consistent evidence that the change in breast density results in a change in the breast cancer risk. For example, breast density generally declines as women age. However, Maskarinec et al. [7] found that women who developed breast cancer had 10.2% higher mammographic densities than controls, but the rate of change in density over a period of more than 20 years was not significantly related to case status. These observations suggest that different risk factors may operate through different pathways for breast cancer development and mammographic density may be an intermediate mediator for some but not all of the known breast cancer risk factors. Today, it is not yet known whether mammographic density can serve as a mediator for breast cancer.

Mediation analysis is often used to assess the relative magnitude of different pathways and mechanisms by which an exposure may affect an outcome, either through a mediator or independent of it. The traditional approach to mediation that is commonly used is the "difference method", which estimates the "proportion mediated (PM)" as a measure of mediated or indirect effect based on the difference in coefficients of the exposure on the outcome from two regression models with and without adjustment for the mediator [5]. However, the traditional approach is often criticized for neither adequately accounting for the strong assumptions that need to be met nor addressing issues of confounding in inferring causal relationships [8-11]. The two regression models may not be valid simultaneously and the estimate can often lie outside the proportion interval (not between 0 and 1). It may lead to biased estimators of indirect effects and incorrect conclusions regarding mediation, especially for logistic regression. These include when there is confounding, when a binary outcome is not rare, or when there is an exposure-mediator interaction [12]. In the last few years, more advance, new approaches for mediation analysis have been developed to address some of these limitations by using the counterfactual framework [9, 13-17]. The causal inference methods for mediation analysis ("causal mediation") emphasizes the identifiability assumptions and conceptual definitions of causal effects, which allows for the decomposition of a total effect into direct and indirect effects, even in models with interactions and nonlinearities. Under the counterfactual framework, the mediated effect is called natural indirect effect (NIE) that compares average outcomes that would be observed if we were to set the exposure as present and change the mediator for each individual from the level it would have been at in the absence of exposure to the level it would have been at in the presence of exposure. Therefore, the NIE captures the effect of exposure on the outcome operating through the mediator. A new approach has recently been developed to statistically assess mediation for a case-control study with a rare outcome [17, 18]. With some modifications, this method can also apply to other study designs. It allows us to identify and separate out the direct and indirect mechanisms of breast cancer development that are acting through or not through mammographic density. A recent study compared the traditional "difference method" and the NIE based on the counterfactual framework and concluded that the "difference method" is always conservative for binary outcomes [19]. It suggested that the "difference method" could be used to provide evidence for the presence of mediation but not for the absence of mediation.

No systematic review has yet been conducted to assess the potential role of mammographic density as a mediator in research on the etiology and prevention of breast cancer for the effects of various exposures on the risk of breast cancer. In this review, we aim to summarize existing scientific evidence on the relevance of mammographic density as a mediator and surrogate marker for breast cancer, with a focus on the statistical approaches and measures for effects of mediation and surrogacy. We examine the extent to which, if any, risk factors for breast cancer influence breast cancer risk through their effects on mammographic density and the extent to which mammographic density can be used as a surrogate endpoint for breast cancer in interventional trials. The summary of evidence helps us outline a picture of potential networks connecting various risk factors to the breast cancer outcome, either through or not through breast density. The results provide insight into the pathways through which various risk factors may affect breast cancer risk, and thereby draws attention to potential paths of intervention.

2.2 METHODS

2.2.1 Search Strategy

A search was conducted using PubMed, Scopus, Web of Knowledge, Google Scholar, and ProQuest Dissertations & Theses (PQDT) from the earliest date available in each database. The search algorithm included all possible combinations of keywords from the following three groups: (i) 'breast cancer', 'breast neoplasm'; (ii) 'mediator', 'mediation', 'biomarker'; (iii) 'breast density', 'mammographic density'. Table 2.1. lists the search terms. We restricted to studies with an English abstract. The most recent search was run on May 15, 2020. We also manually checked the bibliography of relevant articles to identify any articles not found using the above online databases.

2.2.2 Inclusion Criteria and Data Extraction

Studies that met all the following criteria were included in the review: 1) consider at least one potential risk factor for breast cancer; 2) examined breast density measures as a mediator for breast cancer risk; 3) used statistical approaches and/or measures for mediation. All relevant studies had their titles and abstracts screened for eligibility. The selected studies were grouped

according to categories of risk/protection factors, and data were extracted into a spreadsheet, which collected data on study characteristics (i.e., reference, program, design, subjects, measure of mammographic density, exposure, key results, and conclusions). Statistics including unadjusted odds ratio, adjusted odds ratio, measures of mediation/surrogacy, and their 95% confidence intervals were exacted if available.

2.2.3 Data Synthesis

Due to the limited number of studies selected and the heterogeneity of the exposures evaluated, undertaking a meta-analysis was deemed to be not appropriate. The analysis is descriptive. In order to evaluate the direction of mediation, the sign of the NIE for each level of exposure was presented based on the rule by Jiang et al. [19]. In order to quantify the mediating effects of breast density, we calculated the proportion explained (PE) index following the Freedman method [20]. Let F denotes a traditional risk factor, M mammographic density, B breast cancer risk, and C a set of baseline covariates. We could fit two logistic regression models for breast cancer on the risk factor, with or without the percentage density, and the baseline covariates:

$$\text{logit}[P(B = 1|F, C)] = \beta_0 + \beta_1 F + \beta_2 C$$

$$\text{logit}[P(B = 1|F, M, C)] = \theta_0 + \theta_1 F + \theta_2 M + \theta_3 C$$

If the coefficients β_1 and θ_1 differ, then some of the effects are considered to be mediated and the proportion explained by the mediation is calculated as using the following formula:

$$PE = \frac{\beta_1 - \theta_1}{\beta_1} = \frac{\log OR_{unadjusted} - \log OR_{adjusted}}{\log OR_{unadjusted}}$$

PE index is a measure of the proportion of the association of the exposure with breast cancer risk that is explained by mammographic density. The PE index finds the difference between the logarithms of the odds ratios of a risk factor in a model that does not adjust for mammographic density and one that does. This difference is then divided by the log of the odds ratio in the model that does not adjust for mammographic density. In this way, a PE index of 1 implies perfect mediation in that the effect of a risk factor entirely disappears after adjusting for density, and a PE of 0 implies the effect is the same whether adjusting or not, indicating no mediation. Ideally, the

associated 95% confidence intervals (95% CI) for PE should be estimated using the Freedman [20, 21] and the Bootstrap method. However, it is not possible to conduct such statistical tests with data reported in the literature. However, the statistical test results will be extracted if available in the article.

2.3 RESULTS

Characteristics of the selected studies

A total of 895 potential references were screened and 22 studies finally met the selection criteria. The studies covered a wide variety of risk/protective factors for the risk of breast cancer. Most studies did not use a formal statistical mediation analysis. Instead, they only checked two important requirements for a variable to be considered as a mediator to determine the possibility of mammography density acting as a mediator. These two criteria, first laid out by Baron and Kenny [5], suggest that an exposure must be associated with both breast density and breast cancer. Because these studies did not take further statistical mediation analysis, they were not included in the current review. Finally, a total of 22 studies were selected. Among these, 20 studies were based on the traditional "difference method", comparing the estimated coefficients of exposure on the risk of breast cancer in linear regressions before and after adjustment for mammographic density. Out of these, only three studies calculated the percent change in the ORs and/or estimated a measure of mediation [22-24], the proportion explained (PE), which is estimated from the difference in coefficients of an exposure from two logistic regression models with or without adjusting for mammographic density, as described by Freedman et al. [20]. Of these three studies, the 95% confidence interval for the PE was available in only one study [22]. Only two studies have used the counterfactual approach for analysis of mediation [25, 26], one of which was conducted as a secondary analysis [25].

Results based on risk factors

Table 2.2 lists the studies examining the risk of breast cancer according to various genetic factors, including a family history of breast cancer in first-degree relatives [22, 25, 27, 28], genetic variants of single nucleotide polymorphisms (SNPs) [23, 29], and race/ethnicity [23]. A family

history of breast cancer is an established risk factor for breast cancer, which was confirmed in four of the included studies [22, 25, 27, 28]. All these studies observed attenuation of the association between a family history of breast cancer and the risk of breast cancer after adjusting for mammographic density. However, the estimated PE indexes were relatively small. The highest PE was reported in a study with about 75% of the women being postmenopausal, which showed that 14% (95% CI, 4-39%) of the association of a first-degree family history of breast cancer with breast cancer risk was explained by PMD [22]. The upper and lower limits of the confidence interval for this PE were estimated by the Bootstrap method using 1,000 samples (95% CI, 4-35%). However, the other three studies [25, 27, 28] showed no more than 11% of the association was mediated by PMD. This indicates that PMD might not be in the pathway for the association between a family history of breast cancer and the risk of breast cancer and if yes, the mediation effect is likely small. Two selected studies identified genetic variants that had a reduced risk from breast cancer, which were also associated with lower mammographic density [30, 31]. After adjusting for mammographic density, the magnitude of the log ORs for the SNPs on breast cancer risk was reduced by 15% and 35% respectively (Table 2.2). The sign of NIE was consistently negative in both studies, suggesting that these two genetic variants display a negative relationship with breast cancer when it acts through mammographic density. That is, they might reduce breast cancer risk partially by decreasing breast density. However, it is unknown if these PE indexes are statistically significant.

Table 2.3 listed studies considering a variety of clinical features of the breast as risk factors for breast cancer, including computerized mammographic parenchymal pattern (MPP) measure [32], mammographic texture resemblance (MTR) [33], breast tissue stiffness [34], history of benign breast disease (BBD)[25, 35], and history of previous biopsy [27, 28]. Adjustment for mammographic density in general attenuated the association of these risk factors with breast cancer (Table 2.3). However, there is a great variation on the estimated proportion explained with most of the PE indexes for history BBD and biopsy ranging from 12% to 73%.

Among postmenopausal women, breast cancer risk increased with increasing BMI (Table 2.4a). Adjusting for mammographic density did not attenuate the association between adult BMI and postmenopausal breast cancer risk. Rather, it substantially strengthened the association by at

least 43% using the log OR scale (Table 2.4a). Similar results were observed for body weight (Table 2.4b). Among premenopausal women, however, the association of anthropometric measures (BMI and weight) with the risk of breast cancer was not consistent, although the overall total effect is largely negative or insignificant (Table 2.4a-2.4b). Adjusting for mammographic density increased the ORs for all the selected studies. A negative association was moved toward the null or even become positive while a positive association was moved further away from the null after further adjustment for mammographic density. Results among all subjects are mixed but the association of anthropometric measures (BMI, weight, and height) with the risk of breast cancer was mostly insignificant or marginally negative before adjustment for density. The magnitude of the ORs generally increased after controlling for mammographic density. Regardless of menopausal status, adjustment for mammographic density consistently moved the odds ratios for the association between these anthropometric measures (BMI, weight, height) and breast cancer in a positive direction. Although the virtual direction of NIE is inconclusive, adding mammographic density to the regression with BMI or weight as a predictor for breast cancer yielded a large PE index. A majority of the PE indexes were much higher than zero and quite a few exceeded 1. Interestingly, two studies have used a 9-figure body size scale to assess body fatness at ages 5, 7, 10, and 20 years [36, 37] and found that greater body fatness in childhood and adolescence was associated with decreased risk of breast cancer (Table 2.4c). Unlike adult BMI, weight, and height, the inverse association between average childhood and adolescent body fatness and breast cancer risk was generally attenuated after adjustment for mammographic density, indicating a potential consistent mediation (Table 2.4c). The sign of NIE is largely negative with a few inconclusive and the magnitude of the PE index could be as low as 3% [36] and as high as 41% [37].

Few studies have investigated the relationship between reproductive factors (parity, age at first live birth, and age at menarche) with breast cancer, with and without adjustment for mammographic density (Table 2.5). Ever parous was associated with a lower risk of breast cancer and adjusting for mammographic density attenuated the association by about 14-52%, indicating a partial mediation. However, the results for parity were largely inconclusive because the total effect was not significant for some of the included studies. Age at first live birth was found in three studies to be a significant risk factor for breast cancer [25, 27, 28], while it is not significant in a

study among subjects from different ethnic groups [24]. Nevertheless, adjusting for mammographic density slightly attenuated the association in the three studies with significant total effects, yielding results of either no mediation or a PE index ranging from 13% to 17%.

Whether mammographic density can serve as a mediator for the association of postmenopausal hormone use and breast cancer was examined by 4 studies [25-27, 38] (Table 2.6). Current use of hormone replacement therapy (HRT) was associated with an increased risk of breast cancer and the association was consistently attenuated after adjustment for breast density. The sign of NIE is generally positive and the PE indexes range from 10% to 37%. In two of these studies, combined use of estrogen and progesterone was found to have a greater association with the risk of breast cancer than estrogen alone [26, 27]. Further adjustment for mammographic density attenuated the association by 10-26%. Two studies attempted to examine the impact of adjustment for mammographic density on the association of circulating levels of sex hormones with breast cancer risk [39, 40]. In both studies, circulating levels of estradiol and testosterone and mammographic density were both found to be statistically significantly and independently associated with postmenopausal breast cancer risk. However, additional adjustment for mammographic density either strengthened or did not affect the association between these circulating sex hormones and the increased risk of breast cancer, except that in one of the two studies, the association was slightly attenuated for total estradiol and testosterone [39, 40]. Only one study examined sex hormone-binding globulin (SHBG), which was negatively associated with breast cancer risk and this inverse association was strengthened after adjustment for percentage density [40]. The effect of plasma carotenoids on breast cancer risk and its relationship with mammographic density were evaluated in one study [41]. Adjusting for mammographic density altered the association in different directions and the results are largely inconclusive about mediation (Table 2.6).

2.4 DISCUSSION

This review systematically reviewed existing evidence on the potential role of mammographic density plays as a mediator for the risk of breast cancer. The results of this analysis suggest that mammographic density might partially mediate the association between some of the

known risk factors and breast cancer risk. The increase in breast cancer risk due to certain exposures may at least in part be attributed to increases in breast density.

Genetic Factors

Breast density is a highly heritable trait while genetic and family history factors are long-established risk factors for breast cancer. Twin studies have shown that additive genetic factors (heritability) were estimated to account for 53% to 63% of the variation in mammographic density in the population, after adjustment for other factors [42]. Results in our review suggest that breast density may partially mediate the genetic associations with breast cancer. All studies consistently observed attenuation of the association between genetic factors and the risk of breast cancer after adjusting for mammographic density. One of the selected studies statistically confirmed the presence of mediation and showed that percent mammographic density explained 14% of the association of family history (at least one affected first-degree relative) with breast cancer risk [43]. Women with a family history of breast cancer have been shown to have higher mammographic density than women without a family history [44, 45], as have women of Ashkenazi Jewish descent [46]. Although nongenetic components shared by relatives may also play a role, the impact of a family history on the risk of breast cancer is primarily attributed to the inheritance of genes. Therefore, efforts have attempted to identify common genetic variants that predict both breast cancer risk and breast density. Two protective genetic variants satisfying these criteria were found to affect breast cancer risk partially by influencing the proportion of dense tissue in the breast [30, 31]. These results agree with a study that suggested that the genetic components that determine breast density overlapped with the genetic components that influence other breast cancer risk factors [47]. These findings provide insights into the mechanisms underlying the observed gene-disease association and highlight a potential biological pathway involving breast density to the genetic etiology of breast cancer.

Breast Characteristics

All risk factors for breast cancer must ultimately exert their influence by an effect upon breast tissue [48]. Mammographic density, expressed as the percentage of the breast showing densities, reflects variations and changes in the tissue composition of the breast. Fat is

radiologically lucent and appears dark on a mammogram. Dense areas, occupied by epithelial and stromal tissue, are radiologically dense and appear light. Histological assessment of the dense and non-dense areas of the breast revealed that the dense tissue has a greater amount of epithelium and stroma, particularly collagen, increased nuclear occupation, lesser fat, and a higher proportion of proliferative disease without atypia than the non-dense breast tissue [49-51]. Breast cancer most commonly develops in the epithelial cells that line the milk ducts and the lobules that supply these ducts with milk. Therefore, the greater the percent mammographic density, the greater the number of cells available for cancer transformation. Mammographic density is also linked to a higher number of cells and an increased amount of collagen, which may exacerbate breast tissue stiffness [34]. Women at higher risk of breast cancer were found to have dense breasts and their mammographic parenchymal patterns tend to be coarser, and lower in contrast than those of the low-risk group [52-54].

The present review found that the association of some histology and properties of the breast tissue and risk of breast cancer were in general attenuated after adjustment for mammographic density, indicating that their effect on breast cancer risk might be partially mediated through mammographic density. Pathological changes in the breast, reflected by histology abnormality and alterations in parenmychal pattern, texture, and stiffness, are phenomena closely related to breast density. Simply having a history of breast biopsy examination was found to be associated with increased breast density [55]. Women with a previous breast biopsy had an average of 6.5% more density than those who had not had a biopsy, likely due to a greater area of dense tissue and smaller nondense area [56]. Previous studies have described a strong association between benign breast disease histology and mammographic density. Women with density in more than 75% of the mammogram, as compared to women with no density, had a 12.2-fold increased risk of hyperplasia without atypia and 9.7-fold increased risk of atypical hyperplasia and/or breast carcinoma in situ [57, 58]. Compared to women with <25% fibroglandular breast tissue density, the relative risk of benign proliferative breast disease for women with ≥25% density was about doubled [59]. A number of recent studies have identified measures of mammographic parenchymal patterns based on various computer-extracted texture features on mammograms as independent predictors of breast cancer risk [60]. Some of these computerized parenchymal pattern measures, focusing on characteristics of fibroglandular densities seen without taking into account the extent of densities,

still demonstrated a moderate correlation with mammographic density [32, 61-63]. Breast tissue stiffness is an important characteristic that was found to be significantly associated with breast cancer [34]. Greater extracellular matrix (ECM) stiffness was observed in the breast tissue of higher mammographic density [64, 65].

These findings suggest that the association between benign breast diseases, abnormal parenmychal pattern, and greater stiffness and breast cancer risk may, at least in part, be attributable to the biological processes in the breast that give rise to elevated breast density that is known to be related to breast cancer risk. For example, women with benign breast disease are at very high risk for future breast cancer if they also have dense breast tissue than if they have less dense breast tissue [35, 66]. Conversely, women found on breast biopsy to have the lowest category of breast density, whose breast tissue is almost entirely fat, were at low risk for future breast cancer even with proliferative benign pathologic diagnosis [66]. This suggests the important role of breast density plays in the increased risk of breast cancer associated with pathological changes in the breast. However, the estimated PE indexes for these risk factors are mostly no more than 25%, indicating that a relatively small proportion was mediated. The wide range of PE may be explained by the fact that the risk factors covered in this category are highly heterogeneous. Nevertheless, since the PE measure is based on the difference method, which is conservative, we cannot rule out the possibility with the presence of mediation. More study is needed to evaluate this category of risk factors by focusing on the extent of mediation, ideally with a statistical test and alternative mediation analysis such as the counterfactual approach.

Anthropometric Measurements

Non-genetic factors, including environmental, lifestyle, and behavioral exposures that are modifiable, have been much studied in relation to breast cancer risk. Several of these are anthropometric measurements such as body weight, height, and adiposity. BMI in childhood and adolescence was inversely associated with the breast cancer risk, possibly via a mechanism partially mediated by mammographic density, as suggested by the consistent attenuation of the association after adjustment for mammographic density. Adult BMI and body weight, on the other hand, appear to affect the risk of breast cancer for pre- and postmenopausal women differentially.

Before menopause, being overweight or obese is associated with a modestly reduced risk of breast cancer [67-71], whereas after menopause, it increases breast cancer risk [68, 69, 72].

Studies summarized in this review showed that adjustment for mammographic density consistently moved the odds ratio for the association between these anthropometric measures (BMI, weight, height) and breast cancer in a positive direction (increased the ORs). The impact of adding mammographic density to the regression is so strong that the overall negative association among premenopausal women was moved close to the null or even become positive. However, the overall positive association between BMI and breast cancer among postmenopausal women was not attenuated after controlling for mammographic density. Rather, it was substantially strengthened. This is in agreement with another study on postmenopausal women, which found that adjusting for breast density either did not change or strengthened the association of BMI with breast cancer overall and of large, advanced-stage, high nuclear grade, estrogen receptor (ER)-positive and -negative invasive breast cancer [73]. This observation indicates the presence of suppression or inconsistent mediation. In epidemiological studies, this is called negative confounding [3, 74]. It was argued that the negative confounding of percent density with BMI was because of a strong positive correlation of BMI with absolute non-dense (fatty) area on the mammogram [75]. This strong correlation may cause multicollinearity but it was found that this was not an issue [76]. Nevertheless, although mediation, confounding, and suppression are conceptually distinct, they share considerable statistical similarities (statistically equivalent) [77]. Therefore, it is reasonable to hypothesize that the mediated effect via density and the direct effect have opposite signs. That is, BMI may have a negative relationship with breast cancer when it acts through mammographic density (the sign of NIE is generally negative), while the effect not acting through (independent of) mammographic density is positive. This hypothesis suggests that the observed negative association between adult BMI and breast cancer among premenopausal women, along with body fatness in childhood and adolescence and breast cancer in all women, may occur because BMI is acting predominantly through its negative indirect effect through breast density. Overweight or obese women tend to have less dense breasts and, because of this, lower risk or no association with premenopausal breast cancer. The mechanism by which breast density is associated with risk is unknown but might be explained by the combined effects of mitogens in younger women such as insulin-like growth factor 1 (IGF1), which leads to cell proliferation in

the breast and increased amounts of fibroglandular tissue where most breast cancers arise. In postmenopausal women, who tend to have less dense breasts than premenopausal women, BMI may have a more direct positive association with breast cancer.

Several possible biologic mechanisms exist by which women with greater adiposity may have additional risk for breast tumor development in postmenopausal women [78]. One of the possible mechanisms through which excess adiposity is thought to favor breast tumor development is a change in endogenous sex hormone metabolism. The elevated breast cancer risk associated with adiposity (BMI, waist and hip circumferences) in postmenopausal women was substantially and moderately reduced by adjusting for concentrations of serum estrogen (fT, E1, E2, fE2,) and sex hormone-binding globulin, respectively, especially for free estradiol [79-81]. However, adjustment for androgen only slightly reduced the BMI-risk relationship, except for free testosterone which led to a modest attenuation in excess risk with adiposity in one study [79-81]. These observations support the hypothesis that the increased risk of breast cancer associated with greater adiposity in postmenopausal women is largely mediated through estrogen levels, particularly bioavailable estradiol, and to a lesser extent free testosterone, but not total androgens. After menopause, adipose tissue becomes the main site of estrogen production by aromatization of androgens [82, 83]. Furthermore, increased adiposity would cause insulin resistance, which in turn lowers the hepatic synthesis and blood levels of SHBG, a protein that effectively binds both estradiol and testosterone, thereby resulting in an increased concentration of bioavailable sex steroid hormone in circulation [84]. This may explain why women with increased adiposity tend to have higher circulating levels of estrogens [85], whereas overweight and obese postmenopausal women with sustained weight loss showed decreases in estrogen concentrations may reduce the peripheral synthesis and circulating levels of estrogens but not of total androgens [80, 86, 87]. BMI was found to be inversely associated with ER+PR+ breast cancer among premenopausal women but positively associated with risk among postmenopausal women, while no association was observed for risk of ER-PR- breast tumors [73, 88, 89]. This suggests that the effect of adiposity on breast cancer risk may be via an estrogen dependent pathway [90].

Reproductive Factors

Breast density is known to decline during a woman's lifetime, particularly in response to menstrual and reproductive events [91]. Women who were nulliparous or had only one child, with an age at first live birth after age 30 tended to exhibit a slower rate of decline in percent mammographic density in comparison with those having more than one child with the first live birth before age 30 [7]. Percent mammographic density dropped by 2.4% (1.4–3.4) on menopausal transition and increased by 2.4% (1.4–3.5) with the use of hormone replacement therapy [92]. Studies showed that nulliparity, late age at first birth, and premenopausal status were associated with increased percent mammographic density [93-97]. However, there were inconsistent findings for age at menarche and duration of breast-feeding. While some studies found a positive association between mammographic density and age at menarche [97] or duration of breastfeeding [96, 98-100], some studies observed no significant associations [7, 94, 98]. Studies summarized in this review showed that the association between reproductive factors (parity, age at firth birth, and breast-feeding) and breast cancer was reduced (by about 12-17%, 16-37%, and 5% respectively) after adjustment for breast density, indicating that mammographic density might mediate, likely in part, the protective effects of greater parity, younger age at first birth, and a longer period of breastfeeding against breast cancer. This is may explain why the higher risk associated with low parity appeared to be stronger among women with high breast density [101]. The protective effect of parity from breast cancer risk was not wholly mediated by a reduction in mammographic density, which is in agreement with the observation that mammographic density was associated with the risk of both steroid receptor-positive and negative subtypes of breast cancer [102, 103], while parity was found to influence the risk of receptor-positive cancer only [104, 105]. Since no formal statistical test of the mediation effect is available, it is inconclusive whether the proportion mediated estimated above is significant or not. Future studies are needed to further address this issue.

Exogenous Hormone Use

Clinical trials have demonstrated that postmenopausal treatment with hormone replacement therapy (HRT), especially combined formulations of estrogen and progestin, is associated with increases in mammographic density and risk of breast cancer [106], whereas

tamoxifen, a selective anti-estrogen drug, has been shown to reduce mammographic density and breast cancer risk [107]. Although the mechanism of action of these exogenous hormones in influencing the risk of breast cancer remains to be determined, it has been hypothesized that circulating levels of hormones are associated with breast density and that mammographic density represents at least, in part, cumulative exposure to estrogens [108]. The overall effect of HRT in these women was found to delay breast involution and prevent loss of breast parenchyma and epithelial cells that typically occurs around the menopause, thus increasing the breast density in a proportion of the treated patients [109, 110]. Women on a combined estrogen-progestin HRT had 2.5- and 3.7-fold higher serum estradiol and estrone levels than nonuser, while the estradiol concentration in nipple aspirate fluid (NAF) was estimated to 18 times higher than that in nonusers and seven times higher than that in premenopausal women [111]. This indicates that exogenous hormone use may have direct influence on local breast tissue.

Published literature on the effects of using exogenous hormones on mammographic density and the risk of breast cancer suggests that mammographic density might be a potential surrogate marker for breast cancer [106, 108]. Only one study by Boyd et al. [38] directly addressed this question using data from three nested case-control studies and found no support for this hypothesis. The results showed that estimates of the risk of breast cancer associated with hormone replacement therapy were either unchanged or slightly reduced, by adjustment for percent density. Thus, the authors concluded that there was no evidence that the increased risk of breast cancer associated with hormone replacement therapy is a consequence of the effect of this therapy on the risk factor of mammographic density. In other words, the pathways that are responsible for the increase in mammographic density following exposure to exogenous hormones, and those that increase the risk of breast cancer independent of mammographic density, are separate and not related causally. However, this conclusion regarding mediation may be worth further consideration for several reasons. First, the third condition proposed in that study requires the potential surrogate marker to mediate the entire relation of the intervention to the disease. That means it does not allow for partial mediation, instead, it requires the exposure and disease to be statistically unrelated once the surrogate is taken into account, which is a very strong criterion. Second, that study did not formally use any measure of mediation to quantify the extent to which mammographic density influences the association between hormone replacement therapy and breast cancer. We estimated the percent

change in the log ORs with adjustment for mammographic density and found that the PE is about 25% for current HRT users when data on all three nested case-control studies were combined (Table 2.5). This suggests the potential presence of partial mediation, although relatively small. Third, the study used the difference method, which is always conservative for binary outcomes hence the results can be used to provide evidence for the presence of mediation but not for the absence of mediation [19].

Endogenous Hormone

Higher circulating levels of both estrogen and androgen are known to increase the risk of breast cancer in both premenopausal [112-115] and postmenopausal women [40, 80, 116-119], whereas SHBG, which binds estradiol and testosterone with high affinity, is associated with a reduced risk of breast cancer by effectively limiting their bioavailability [120, 121]. The biologic mechanism by which estrogens and androgens are associated with increased breast cancer risk remains unclear, although they are closely related. Androgens may act directly, promoting breast cell growth via binding to the androgen receptor, or indirectly, via conversion to estrogens in adipose tissue, either peripherally or locally in the breast [122, 123]. It was argued that the contribution of androgens to breast cancer risk might be largely through their role as estrogen precursors. This is because the association between androgen (testosterone) levels and breast cancer risk decreased substantially after adjusting for estrone sulfate and slightly after adjusting for total estradiol [80, 116, 124-126]. On the other hand, adjustment for androgen levels only mildly attenuated the relative risk of breast cancer associated with estrogens [80, 116]. Since the risk remained significant after adjustment, it indicates that androgens may also act through an independent mechanism in addition to increasing estrogen levels.

The observed associations of mammographic density with menstrual and reproductive factors, along with exogenous sex hormone use, support that endogenous sex steroids may also increase breast cancer risk through their effect on breast density. However, it is unclear to what extent their effects on breast cancer risk are independent of the effect of mammographic density or to what extent density is a reflection of underlying hormone levels. In the current review, we identified two studies that attempted to address this question but the results were largely inconclusive about mediation. Adjustment for mammographic density mostly either strengthened

or did not affect the association between these circulating sex hormones and increased risk of breast cancer. On the other hand, both studies showed that the elevated risk of postmenopausal breast cancer associated with increased mammographic density became stronger after adjustment for sex hormones or SHBG, more so for those in the free available form [39, 40]. It is possible that endogenous hormones in the free form are acting differently or with greater bioactivity than those in bounded form. These observations suggest that the possible mechanism underlying the association between endogenous sex hormones and postmenopausal breast cancer risk is complex and not well understood. One potential explanation for this might be the presence of inconsistent mediation or suppression. This suggests that if the indirect pathway through breast density exists, then such intermediate partly counters the positive associations of free estradiol and testosterone, and the adverse association of SHBG with breast cancer risk. That is, free estradiol and testosterone are likely to be negatively and SHBG positively associated with mammographic density.

However, previous studies on the association between sex hormone levels and breast density have been largely inconsistent. While some studies reported that levels of estradiol, prolactin, progesterone, testosterone, premenarchal DHEAS (dehydroepiandrosterone sulfate), and SHBG were positively associated with mammographic density among premenopausal women [127-131], others found either no association of plasma estradiol, progesterone, non-SHBG-bound testosterone, and SHBG, or negative association of androgens testosterone, androstenedione, and dehydroepiandrosterone sulfate with premenopausal breast density [127, 129, 132, 133]. Among postmenopausal women, higher blood levels of endogenous estrogens and androgens (estrone, estradiol, bioavailable estradiol, prolactin, and progesterone) were shown to be related to greater mammographic density, even after adjustment for BMI [132, 134-138]. But in other studies, circulating levels of estrone, estradiol, free estradiol, testosterone, free testosterone, androstenedione, and dehydroepiandrosterone were found to be negatively [40, 132, 135, 139-141] and sex SHBG [135, 136, 138, 142] to be positively associated with percentage density. In a recent study on postmenopausal women, the ratio of urinary parent estrogens (estrone and estradiol) to all their metabolites (methylated catechols, 2-methoxyestrone, and 4-methoxyestrone) was found to be positively associated with percent mammographic density and dense area, which did not differ markedly by 2-, 4-, and 16-hydroxylation metabolic pathways. This suggests that increased hydroxylation of parent estrogens may protect against breast cancer

through a pathway involving breast density [143]. However, this association of estrogens and MD is maintained only among postmenopausal women with recent or sustained exposure to higher levels of circulating estrogens (such as occurs close to the time of menopause or among obese women [143].

Nevertheless, the association between sex hormone levels and mammographic density is strongly influenced by BMI. Most of these hormone-density associations (total/free/bioavailable estradiol, estrone, estrone sulfate, total/free/bioavailable testosterone, or SHBG levels, prolactin) were substantially weakened or eliminated with further adjustment for BMI in both premenopausal [127] and postmenopausal women [40, 108, 135, 138, 140, 144]. These results suggest that if the effect of circulating sex hormone levels to breast cancer is mediated by mammographic density, then they act through a pathway involving obesity. This agrees with a study that used statistical mediation analysis based on the difference method, which found that bodyweight mediated over 50% of the association of progesterone, SHBG, and E2 with percent mammographic density in premenopausal women [131]. Meantime, this study found no support for the hypothesis that any of the hormones mediated the association of weight with percentage density [131].

Even if BMI mediated a large part of the association between circulating hormones with breast density, it is likely to mediate only a small proportion of the effect of circulating hormone on breast cancer risk because only part of this association is acting through breast density. Adjustment for BMI resulted in little change in the risk estimates of breast cancer for different levels of androgens, estrogens, or SHBG in postmenopausal women, suggesting a possible causal role of sex steroids in breast cancer is independent of postmenopausal obesity [40, 79-81, 118]. This is consistent with the finding that the reduced risk of breast cancer associated with greater hydroxylation of parent estrogens did not vary by >10% with and without adjustment for BMI, suggesting that adiposity is neither a confounder of the association nor is it on the causal pathway of estrogen metabolism to mammographic density [143]. Adjusting for both BMI and height simultaneously was found to reduce the ORs for postmenopausal breast cancer associated with top quintile of oestradiol, oestrone, and androgens by 17%, 18%, and 7–13% respectively [124].

While mammographic density affected risks of both estrogen- and progesterone-receptor positive (ER+/ PR+) and negative (ER-/PR-) breast cancers [102, 103], hormone replacement therapy (HRT) use and circulating levels of sex steroid hormones tended to be more strongly associated with risk of receptor-positive (ER+/PR+) breast tumors [88, 108, 114, 117, 119]. Furthermore, clinical trials showed that treatment with tamoxifen [145] or raloxifene [146] appeared to be effective in reducing the risk of estrogen-receptor–positive (ER+), but not receptor-negative (ER-) breast tumors. Therefore, it was hypothesized that circulating sex hormone levels and mammographic density are independent risk factors for postmenopausal breast cancer and that they may increase breast cancer risk through different mechanisms.

Limitations

There are several limitations to the current review. First, this review is limited by the relatively small number of studies that have evaluated the potential role of mammographic density as a mediator. The risk factors for breast cancer covered were of such a great variety that it is impossible to conduct a meta-analysis. Therefore, the results must be interpreted with caution. Second, although the studies encompass a wide range of risk factors, it should be noted that the studies examined in this review were rarely designed to directly address the specific mediation question being investigated here. Third, all except two studies used the "difference method", which has been criticized for lacking a causal interpretation and being conservative regarding mediation for binary outcomes. The proportion mediated tends to be underestimated. It can be used to provide evidence for the presence of mediation but not for the absence of mediation. While the "difference method" is commonly used, the question remains whether density mediates the effects of risk factors for breast cancer. Given that there are only two studies that have conducted causal mediation analysis, the role of mammographic density linking a risk factor and breast cancer risk remains unclear.

Despite these limitations, the data summarized in this review do provide insights for us to generate important hypotheses regarding the potential mediation role of mammographic density in a wide range of factors known to be related to breast cancer risk. Therefore, further studies are needed to test the hypothesis that some risk factors may affect breast cancer risk through a pathway

via mammographic density. The findings can help us to better understand the biological mechanisms involving mammographic density to the etiology of breast cancer.

2.5 CONCLUSIONS

In conclusion, some evidence supported the hypothesis of mediating pathways from a risk factor such as HRT to breast cancer through mammographic density measures. Very few studies have used statistical mediation analyses to examine the effect of known risk factors on breast cancer risk through breast density. The available evidence is not enough to make a conclusion about the potential mediation effect of breast density. Despite a lack of sufficient evidence, available data based on the "difference method" implies that mammographic density may play a role in the effects of some breast cancer risk factors. The association between many of the known risk factors on breast cancer is likely in part, although not wholly, mediated by mammographic density. This is especially true for the effect of adiposity since adjustment for mammographic density substantially altered its association with the risk of breast cancer. Further research is needed to address the hypothesis generated in this review and to statistically examine the potential role of mammographic density as a mediator in the etiology of breast cancer, not only based on the traditional "difference method" but also based on the newly developed techniques such as the counterfactual approach.

TABLES

Table 2.1. Search strategies

Search #	Search using title, abstract, and keywords
#1	(((mammography OR mammographic) AND (density OR densities)) OR "breast density" OR "breast densities" OR "percent density" OR "percent densities") AND (mediat* OR surrogat*)
#2	(((mammography OR mammographic) AND (density OR densities)) OR "breast density" OR "breast densities" OR "percent density" OR "percent densities") AND ("adjusted for" OR "adjust for" OR "adjusts for" OR "adjusting for" OR "adjustment for" OR "controlled for" OR "controlling for" OR "accounting for")
#3	#1 OR #2

Table 2.2. Changes in the OR/RRs of genetic factors by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE/ PM
2011	Lindström	806/784	NHS	rs10995190 in ZNF365	0.88	(0.72-1.07)	0.93	(0.76-1.14)	negative	0.43
2011	Lindström	518/742	SASBAC	rs10995190 in ZNF365	0.91	(0.73-1.14)	0.93	(0.75-1.17)	negative	0.23
2011	Lindström	783/907	MCBCS	rs10995190 in ZNF365	0.78	(0.65-0.95)	0.85	(0.69-1.03)	negative	0.35
2011	Lindström	2107/2433	Combined	rs10995190 in ZNF365	0.85	(0.76-0.96)	0.90	(0.80-1.01)	negative	0.35
2014	Fejerman	304/809	Mexican	rs140068132	0.73	(0.55-0.98)	0.77	(0.58-1.03)	negative	0.15
2014	Fejerman	304/809	Mexican	Indigenous American Ancestry	0.34	(0.16-0.73)	0.34	(0.16-0.74)	negative	0.01
2014	Fejerman	304/809	Mexican	African Ancestry	1.03	(0.08-13.11)	0.81	(0.06-10.59)	inconclusive	8.61
2010	Martin	926/978	~75% postmenopausal	# of 1st° relatives n=0	1.00	Referent	1.00	Referent	~	~
2010	Martin	207/165	~75% postmenopausal	# of 1st° relatives n=1	1.37	(1.10-1.72)	1.31	(1.04-1.65)	positive	0.14
2010	Martin	31/15	~75% postmenopausal	# of 1st° relatives n≥2	2.45	(1.30-4.62)	2.25	(1.19-4.27)	positive	0.10
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Family history of breast cancer: Yes vs no	1.47	(1.07,2.01)	1.46	(1.06,2.00)	positive	2%
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Family history of breast cancer: Yes vs no	1.45	(1.15,1.85)	1.45	(1.14,1.84)	positive	1%
2018	Rice	1083/3190	NHS/NHSII, premenopausal at mammogram	Family history of breast cancer: Yes vs no	1.59	(1.30,1.94)	1.55	(1.27,1.90)	positive	5%
2018	Rice	2188/5669	NHS/NHSII, postmenopausal at mammogram	Family history of breast cancer: Yes vs no	1.58	(1.39,1.80)	1.58	(1.39,1.80)	positive	1%
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: <20 yrs, n=0	1.00	Referent	1.00	Referent	~	~
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: <20 yrs, n=1	2.89	(1.82-4.57)	2.80	(1.77-4.43)	positive	0.03
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: <20 yrs, n=2+	8.33	(3.32-20.90)	7.83	(3.13-19.60)	positive	0.03
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 20-24 yrs, n=0	1.27	(1.09-1.48)	1.22	(1.05-1.42)	positive	0.17

Table 2.2. Changes in the OR/RRs of genetic factors by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE/ PM
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 20-24 yrs, n=1	2.98	(2.11-4.21)	2.80	(1.98-3.95)	positive	0.06
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 20-24 yrs, n=2+	6.99	(3.86-12.70)	6.40	(3.54-11.60)	positive	0.05
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 25-29 yrs, n=0	1.62	(1.20-2.18)	1.50	(1.10-2.03)	positive	0.16
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 25-29 yrs, n=1	3.08	(2.18-4.36)	2.80	(1.97-3.98)	positive	0.08
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 25-29 yrs, n=2+	5.87	(3.60-9.57)	5.24	(3.21-8.56)	positive	0.06
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 30+ yrs, n=0	2.05	(1.31-3.22)	1.83	(1.16-2.89)	positive	0.16
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 30+ yrs, n=1	3.18	(2.00-5.07)	2.80	(1.75-4.49)	positive	0.11
2005	Tice	81,777	diverse racial groups	Age at 1st birth, # of 1st° relatives: 30+ yrs, n=2+	4.93	(2.43-9.99)	4.28	(2.10-8.74)	positive	0.09

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated
PE was estimated using the following formula: PE = $[\beta_{(unadjusted)}-\beta_{(adjusted)}]/\beta_{(unadjusted)}$
PM was reported in the paper, denoted as a percentage.

Table 2.3. Changes in the OR/RRs of clinical features of the breast by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE / PM
2011	Wei	41/174	Training and test set	MPP C1	1.00	Referent	1.00	Referent	~	~
2011	Wei	28/50	Training and test set	MPP C2	2.62	NA	2.40	(1.28-4.45)	positive	0.09
2011	Wei	67/22	Training and test set	MPP C3	13.91	NA	13.38	(7.12-25.15)	positive	0.01
2011	Wei	21/87	Training set	MPP C1	1.00	Referent	1.00	Referent	~	~
2011	Wei	19/35	Training set	MPP C2	2.65	NA	2.37	(1.04-5.36)	positive	0.11
2011	Wei	41/14	Training set	MPP C3	13.91	NA	13.95	(5.93-32.85)	inconclusive	0.00
2011	Wei	20/87	Test set C3	MPP C1	1.00	Referent	1.00	Referent	~	~
2011	Wei	9/15	Test set C4	MPP C2	2.87	NA	2.82	(1.04-7.64)	positive	0.02
2011	Wei	26/8	Test set C5	MPP C3	14.00	NA	13.89	(6.53-49.05)	positive	0.00
2014	Nielsen	226/442	Study S2 trained on S1	MTR T1 quartiles Q1	1.00	Referent	1.00	Referent	~	~
2014	Nielsen	226/443	Study S2 trained on S1	MTR T1 quartiles Q2	1.40	(0.80-2.30)	1.04	(0.59-1.81)	positive	0.88
2014	Nielsen	226/444	Study S2 trained on S1	MTR T1 quartiles Q3	1.30	(0.70-2.20)	0.95	(0.52-1.74)	inconclusive	1.20
2014	Nielsen	226/445	Study S2 trained on S1	MTR T1 quartiles Q4	2.20	(1.40-3.60)	1.84	(1.10-3.07)	positive	0.23
2014	Nielsen	226/446	Study S2 trained on S1	MTR T1 (OR per one SD)	1.39	(1.17-1.66)	1.36	(1.13-1.62)	positive	0.07
2014	Boyd	362/656	most postmenopausal	Stiffness IQR (interquartile range)	1.24	(1.05-1.46)	1.21	(1.03-1.43)	positive	0.11
2001	Byrne	62/94	~85% postmenopausal	Nonproliferative benign disease	1.00	Referent	1.00	Referent	~	~
2001	Byrne	198/223	~85% postmenopausal	Proliferative disease without atypia	1.30	(0.90-1.90)	1.30	(0.90-1.90)	positive	0.00
2001	Byrne	58/41	~85% postmenopausal	Atypical hyperplasia	2.20	(1.30-3.60)	2.10	(1.30-3.60)	positive	0.06
2001	Byrne	29/52	~85% postmenopausal	Benign histology, Other	0.80	(0.50-1.40)	0.90	(0.50-1.70)	negative	0.53
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	History of biopsy-confirmed BBD Yes vs no	2.04	(1.59,2.62)	1.81	(1.40,2.32)	positive	17%**
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	History of biopsy-confirmed BBD Yes vs no	1.29	(1.04,1.61)	1.19	(0.95,1.48)	positive	33%*
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	History of unconfirmed BBD	1.12	(0.89,1.41)	1.03	(0.82,1.30)	positive	73%

Table 2.3. Changes in the OR/RRs of clinical features of the breast by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE / PM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	History of unconfirmed BBD	1.19	(0.95,1.48)	1.13	(0.90,1.41)	positive	29%
2018	Rice	1098/3183	NHS/NHSII, premenopausal at mammogram	Previous breast biopsy yes versus no	1.76	(1.48,2.10)	1.60	(1.34,1.91)	positive	17%**
2018	Rice	2202/5546	NHS/NHSII, postmenopausal at mammogram	Previous breast biopsy yes versus no	1.50	(1.34,1.69)	1.36	(1.21,1.53)	positive	24%**
2005	Tice	81,777	diverse racial groups	Age<50 years, No previous biopsy	1.00	Referent	1.00	Referent	~	~
2005	Tice	81,777	diverse racial groups	Age<50 years, Previous biopsy	1.22	(0.82-1.82)	1.19	(0.80-1.78)	positive	0.13
2005	Tice	81,777	diverse racial groups	Age<50 years, >1 previous biopsy	1.49	(0.67-3.31)	1.42	(0.64-3.16)	positive	0.12
2005	Tice	81,777	diverse racial groups	Age≥50 years, No previous biopsy	1.00	Referent	1.00	Referent	~	~
2005	Tice	81,777	diverse racial groups	Age≥50 years, Previous biopsy	1.24	(0.99-1.56)	1.19	(0.94-1.50)	positive	0.19
2005	Tice	81,777	diverse racial groups	Age≥50 years, >1 previous biopsy	1.54	(0.97-2.45)	1.41	(0.88-2.24)	positive	0.20

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated; NA: not available; MPP: mammographic parenchymal pattern; MTR: mammographic texture resemblance; BBD: benign breast disease
PE was estimated using the following formula: $PE = [\beta_{(unadjusted)}-\beta_{(adjusted)}]/\beta_{(unadjusted)}$
PM was reported in the selected studies, denoted as a percentage
* $p < 0.05$, ** $p < 0.01$

Table 2.4a. Changes in the OR/RRs of BMI by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure BMI (kg/m^2)	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE /PM
2004	Vacek	24006	Premenopausal	<22.0	1.00	Referent	1.00	Referent	~	~
2004	Vacek	24006	Premenopausal	22.0-24.9	0.66	(0.50-0.88)	0.71	(0.53-0.94)	negative	0.18
2004	Vacek	24006	Premenopausal	25.0–27.4	0.68	(0.48-0.95)	0.77	(0.55-1.09)	negative	0.32
2004	Vacek	24006	Premenopausal	27.5–29.9	0.66	(0.44-0.99)	0.79	(0.53-1.20)	negative	0.43
2004	Vacek	24006	Premenopausal	≥ 30.0	0.64	(0.47-0.88)	0.85	(0.61-1.20)	negative	0.64
2006	Boyld	86/64	Premenopausal	≤21.79	1.00	Referent	1.00	Referent	~	~
2006	Boyld	54/59	Premenopausal	(21.79-23.30)	0.69	(0.40-1.10)	0.88	(0.5-1.5)	negative	0.66
2006	Boyld	49/46	Premenopausal	(23.30-25.02)	0.79	(0.50-1.30)	1.13	(0.60-2.00)	inconclusive	1.52
2006	Boyld	42/46	Premenopausal	(25.02-27.64)	0.68	(0.40-1.20)	1.06	(0.60-1.90)	inconclusive	1.15
2006	Boyld	51/52	Premenopausal	>27.64	0.76	(0.50-1.30)	1.47	(0.80-2.70)	inconclusive	2.40
2006	Boyld	162 pairs	Premenopausal	BMI (continuous)	0.96	(0.91-1.02)	1.01	(0.95-1.07)	inconclusive	1.14
2011	Harris	19/46	Premenopausal	<20b	0.86	(0.47-1.58)	0.75	(0.40-1.38)	inconclusive	-0.91
2011	Harris	77/151	Premenopausal	20–22.4b	1.00	Referent	1.00	Referent	~	~
2011	Harris	60/146	Premenopausal	22.5–24.9b	0.85	(0.56-1.28)	1.04	(0.68-1.60)	inconclusive	1.24
2011	Harris	48/23	Premenopausal	25–27.4b	1.08	(0.68-1.71)	1.59	(0.97-2.59)	inconclusive	-5.03
2011	Harris	23/26	Premenopausal	27.5–29.9b	1.75	(0.92-3.33)	2.86	(1.44-5.68)	inconclusive	-0.88
2011	Harris	31/100	Premenopausal	≥30b	0.64	(0.38-1.06)	1.28	(0.72-2.30)	inconclusive	1.55
2000	Lam	298/1241	Postmenopausal	<22.0	1.00	Referent	1.00	Referent	~	~
2000	Lam	298/1241	Postmenopausal	22.0-24.9	1.20	(0.80-2.00)	1.40	(0.90-2.30)	inconclusive	-0.85
2000	Lam	298/1241	Postmenopausal	25.0–27.4	1.30	(0.80-2.20)	1.60	(0.90-2.70)	inconclusive	-0.79
2000	Lam	298/1241	Postmenopausal	27.5–29.9	1.30	(0.70-2.10)	1.60	(0.90-2.70)	inconclusive	-0.79
2000	Lam	298/1241	Postmenopausal	≥ 30.0	1.90	(1.20-3.00)	2.50	(1.60-4.10)	inconclusive	-0.43
2004	Vacek	36867	Postmenopausal	<22.0	1.00	Referent	1.00	Referent	~	~
2004	Vacek	36867	Postmenopausal	22.0-24.9	1.09	(0.87-1.37)	1.17	(0.94-1.45)	inconclusive	-0.82
2004	Vacek	36867	Postmenopausal	25.0–27.4	1.07	(0.84-1.37)	1.25	(0.99-1.57)	inconclusive	-2.30
2004	Vacek	36867	Postmenopausal	27.5–29.9	1.18	(0.91-1.53)	1.43	(1.12-1.83)	inconclusive	-1.16

Table 2.4a. Changes in the OR/RRs of BMI by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure BMI (kg/m^2)	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE /PM
2004	Vacek	36867	Postmenopausal	≥ 30.0	1.19	(0.95-1.50)	1.54	(1.23-1.93)	inconclusive	-1.48
2006	Boyld	159/168	Postmenopausal	≤21.79	1.00	Referent	1.00	Referent	~	~
2006	Boyld	164/158	Postmenopausal	(21.79-23.30)	1.05	(0.80-1.40)	1.16	(0.80-1.60)	inconclusive	-2.04
2006	Boyld	159/174	Postmenopausal	(23.30-25.02)	0.95	(0.70-1.30)	1.13	(0.80-1.60)	inconclusive	3.38
2006	Boyld	170/178	Postmenopausal	(25.02-27.64)	1.02	(0.80-1.40)	1.28	(0.90-1.80)	inconclusive	-11.47
2006	Boyld	180/169	Postmenopausal	>27.64	1.17	(0.90-1.60)	1.67	(1.20-2.30)	inconclusive	-2.27
2006	Boyld	727 pairs	Postmenopausal	BMI (continuous)	1.02	(0.99-1.04)	1.05	(1.02-1.03)	inconclusive	-1.81
2006	Boyld	245/232	All subjects	≤21.79	1.00	Referent	1.00	Referent	~	~
2006	Boyld	218/217	All subjects	(21.79-23.30)	0.93	(0.70-1.20)	1.07	(0.80-1.40)	inconclusive	1.93
2006	Boyld	208/220	All subjects	(23.30-25.02)	0.89	(0.70-1.20)	1.12	(0.80-1.50)	inconclusive	1.97
2006	Boyld	212/224	All subjects	(25.02-27.64)	0.91	(0.70-1.20)	1.21	(0.90-1.60)	inconclusive	3.02
2006	Boyld	231/221	All subjects	>27.64	1.04	(0.80-1.40)	1.60	(1.20-2.20)	inconclusive	-10.98
2006	Boyld	1,114 pairs	All subjects	BMI (continuous)	1.01	(0.99-1.03)	1.04	(1.02-1.06)	inconclusive	-3.90
2011	Harris	185/339	All subjects	BMI at Age 18: <18.5	0.97	(0.79-1.20)	0.92	(0.75-1.14)	inconclusive	-1.74
2011	Harris	314/559	All subjects	BMI at Age 18: 18.5–19.9	1.03	(0.87-1.23)	0.96	(0.81-1.14)	inconclusive	2.38
2011	Harris	616/1124	All subjects	BMI at Age 18: 20–22.4	1.00	Referent	1.00	Referent	~	~
2011	Harris	238/449	All subjects	BMI at Age 18: 22.5–24.9	0.96	(0.80-1.16)	1.07	(0.89-1.30)	inconclusive	2.66
2011	Harris	99/260	All subjects	BMI at Age 18: ≥25	0.68	(0.52-0.87)	0.84	(0.65-1.09)	negative	0.55
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	BMI, Per 5-unit increase	1.03	(0.92,1.16)	1.22	(1.07,1.39)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	BMI, Per 5-unit increase	1.05	(0.95,1.16)	1.17	(1.05,1.30)	inconclusive	NM
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	BMI at Age 18, Per 5-unit increase	0.80	(0.65, 0.97)	0.96	(0.78,1.19)	negative	82%*

Table 2.4a. Changes in the OR/RRs of BMI by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure BMI (kg/m^2)	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE /PM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	BMI at Age 18, Per 5-unit increase	0.88	(0.74,1.05)	1.00	(0.83,1.19)	negative	98%
2018	Rice	1105/3192	NHS/NHSII, premenopausal at mammogram	BMI, Per 5-unit increase	0.98	(0.92,1.05)	1.20	(1.10,1.29)	inconclusive	NM
2018	Rice	2287/5690	NHS/NHSII, postmenopausal at mammogram	BMI, Per 5-unit increase	1.14	(1.09,1.19)	1.33	(1.26,1.40)	inconclusive	NM
2014	Andersen	12640	All subjects	BMI at age 7	0.91	(0.83-0.99)	0.97	(0.88-1.06)	negative	0.68
2014	Andersen	12887	All subjects	BMI at age 8	0.94	(0.86-1.02)	1.01	(0.92-1.11)	inconclusive	1.16
2014	Andersen	12968	All subjects	BMI at age 9	0.91	(0.83-1.00)	0.99	(0.90-1.09)	negative	0.89
2014	Andersen	13014	All subjects	BMI at age 10	0.92	(0.83-1.01)	1.01	(0.92-1.10)	inconclusive	1.12
2014	Andersen	13045	All subjects	BMI at age 11	0.95	(0.87-1.04)	1.05	(0.95-1.14)	inconclusive	1.95
2014	Andersen	13050	All subjects	BMI at age 12	0.92	(0.85-1.01)	1.02	(0.93-1.12)	inconclusive	1.24
2014	Andersen	13002	All subjects	BMI at age 13	0.92	(0.84-1.00)	1.01	(0.93-1.11)	inconclusive	1.12
2014	Fejerman	304/809	All subjects (Mexican)	BMI (continuous)	0.95	(0.93-0.98)	0.97	(0.94-1.00)	negative	0.40

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated; NA: not available; NM: not mediated

PE was estimated using the following formula: $PE = [\beta_{(unadjusted)} - \beta_{(adjusted)}]/\beta_{(unadjusted)}$

PM was reported in the selected studies, denoted as a percentage

* $p < 0.05$, ** $p < 0.01$

Table 2.4b. Changes in the OR/RRs of weight/height by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure Somatotype	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
1984	Brisson	23/63	Premenopausal	Body weight (kg) <55	1.00	Referent	1.00	Referent	~	~
1984	Brisson	39/113	Premenopausal	Body weight (kg) 55-64	0.90	(0.50-1.70)	1.10	(0.60-2.20)	inconclusive	1.90
1984	Brisson	28/49	Premenopausal	Body weight (kg) 65-74	1.50	(0.70-3.10)	2.60	(1.20-5.90)	inconclusive	-1.36
1984	Brisson	14/31	Premenopausal	Body weight (kg) ≥75	1.20	(0.50-2.80)	2.70	(1.00-7.20)	inconclusive	-4.45
2006	Boyld	162 pairs	Premenopausal	Weight (kg)	0.99	(0.97-1.01)	1.00	(0.98-1.03)	inconclusive	1.30
1984	Brisson	46/96	Postmenopausal	Body weight (kg) <55	1.00	Referent	1.00	Referent	~	~
1984	Brisson	90/172	Postmenopausal	Body weight (kg) 55-64	1.10	(0.70-1.80)	1.30	(0.80-2.10)	inconclusive	-1.75
1984	Brisson	64/88	Postmenopausal	Body weight (kg) 65-74	1.50	(0.90-2.60)	2.10	(1.20-3.70)	inconclusive	-0.83
1984	Brisson	55/73	Postmenopausal	Body weight (kg) ≥75	1.60	(1.00-2.70)	2.60	(1.40-4.60)	inconclusive	-1.03
2000	Lam	298/1241	Postmenopausal	weight ≤63 kg	1.00	Referent	1.00	Referent	~	~
2000	Lam	298/1241	Postmenopausal	63.1–70.0 kg	1.50	(1.00-2.30)	1.60	(1.10-2.50)	inconclusive	-0.16
2000	Lam	298/1241	Postmenopausal	70.1–81 kg	1.60	(1.10-2.40)	1.90	(1.20-2.80)	inconclusive	-0.37
2000	Lam	298/1241	Postmenopausal	weight >81 kg	1.70	(1.20-2.60)	2.10	(1.30-3.20)	inconclusive	-0.40
2006	Boyld	727 pairs	Postmenopausal	Weight (kg)	1.01	(1.00-1.02)	1.02	(1.01-1.03)	inconclusive	-1.20
1984	Brisson	69/160	All subjects	Body weight (kg) <55	1.00	Referent	1.00	Referent	~	~
1984	Brisson	129/285	All subjects	Body weight (kg) 55-64	1.10	(0.70-1.50)	1.30	(0.90-1.80)	inconclusive	-1.75
1984	Brisson	94/137	All subjects	Body weight (kg) 65-74	1.60	(1.10-2.40)	2.30	(1.50-3.70)	inconclusive	-0.77
1984	Brisson	70/104	All subjects	Body weight (kg) ≥75	1.50	(1.00-2.40)	2.70	(1.60-4.40)	inconclusive	-1.45
2006	Boyld	1,114 pairs	All subjects	Weight (kg)	1.00	(1.00-1.01)	1.01	(1.01-1.02)	inconclusive	-3.34
2014	Andersen	8,271	All subjects	Birth weight (g)	0.89	(0.75-1.06)	0.88	(0.74-1.05)	inconclusive	-0.10
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Weight change since 18, Per 20-lb increase	1.03	(0.96,1.12)	1.16	(1.06,1.26)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Weight change since 18, Per 20-lb increase	1.03	(0.96,1.10)	1.11	(1.03,1.19)	inconclusive	NM
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Height Per 3-inch increase	1.14	(1.01,1.28)	1.14	(1.01,1.29)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Height Per 3-inch increase	0.95	(0.85,1.06)	0.96	(0.86,1.07)	negative	22%

Table 2.4b. Changes in the OR/RRs of weight/height by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure Somatotype	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2018	Rice	1060/3018	NHS/NHSII, premenopausal at mammogram	Height Per 3-inch increase	1.02	(0.94,1.11)	1.04	(0.96,1.13)	inconclusive	NM
2018	Rice	2114/4938	NHS/NHSII, postmenopausal at mammogram	Height Per 3-inch increase	1.06	(0.99,1.12)	1.08	(1.02,1.15)	inconclusive	NM
1984	Brisson	69/94	All subjects	Body height (cm) <155	1.00	Referent	1.00	Referent	~	~
1984	Brisson	99/208	All subjects	Body height (cm) 155-159	0.60	(0.40-0.90)	0.60	(0.40-0.90)	inconclusive	0.00
1984	Brisson	90/197	All subjects	Body height (cm) 160-164	0.60	(0.40-0.90)	0.50	(0.30-0.80)	inconclusive	-0.36
1984	Brisson	104/187	All subjects	Body height (cm) ≥165	0.80	(0.50-1.20)	0.70	(0.40-0.90)	inconclusive	-0.60
2014	Fejerman	304/809	All subjects (Mexican)	Height (cm)	1.03	(1.00-1.05)	1.02	(1.00-1.05)	positive	0.12
2014	Andersen	12,636	All subjects	Height (cm) age 7	1.06	(0.98-1.14)	1.06	(0.99-1.15)	inconclusive	0.00
2014	Andersen	12,882	All subjects	Height (cm) age 8	1.05	(0.98-1.14)	1.06	(0.98-1.14)	inconclusive	-0.19
2014	Andersen	12,963	All subjects	Height (cm) age 9	1.05	(0.97-1.13)	1.06	(0.98-1.14)	inconclusive	-0.19
2014	Andersen	13,011	All subjects	Height (cm) age 10	1.05	(0.97-1.13)	1.06	(0.98-1.14)	inconclusive	-0.19
2014	Andersen	13,039	All subjects	Height (cm) age 11	1.05	(0.98-1.14)	1.07	(0.99-1.15)	inconclusive	-0.39
2014	Andersen	13,044	All subjects	Height (cm) age 12	1.07	(0.99-1.15)	1.08	(1.01-1.16)	inconclusive	-0.14
2014	Andersen	12,991	All subjects	Height (cm) age 13	1.08	(1.00-1.16)	1.09	(1.01-1.17)	inconclusive	-0.12
2006	Boyld	1,114 pairs	All subjects	Height (cm)	1.00	(0.99-1.01)	1.00	(0.99-1.01)	positive	0.14
2006	Boyld	162 pairs	Premenopausal	Height (cm)	1.00	(0.97-1.03)	1.01	(0.97-1.04)	inconclusive	-21.00
2006	Boyld	727 pairs	Postmenopausal	Height (cm)	1.01	(0.99-1.03)	1.01	(0.99-1.03)	positive	0.12

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated; NM: not mediated

PE was estimated using the following formula: $PE = [\beta_{(unadjusted)} - \beta_{(adjusted)}]/\beta_{(unadjusted)}$

PM was reported in the paper, denoted as a percentage.

Table 2.4c. Changes in the OR/RRs of childhood and adolescent body fatness by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2010	Jingmei Li	902/862	postmenopausal, all	Somatotype at age 7	Lean (1-2)	1.00	Referent	1.00	Referent	~	~
2010	Jingmei Li	353/428	postmenopausal, all	Somatotype at age 7	Medium (3-4)	0.79	(0.66-0.94)	0.79	(0.66-0.94)	inconclusive	0.00
2010	Jingmei Li	79/108	postmenopausal, all	Somatotype at age 7	Large (5-9)	0.66	(0.48-0.90)	0.67	(0.49-0.92)	negative	0.04
2010	Jingmei Li	510/862	postmenopausal, ER-positive	Somatotype at age 7	Lean (1-2)	1.00	Referent	1.00	Referent	~	~
2010	Jingmei Li	200/428	postmenopausal, ER-positive	Somatotype at age 7	Medium (3-4)	0.79	(0.65-0.98)	0.80	(0.65-0.98)	negative	0.05
2010	Jingmei Li	49/108	postmenopausal, ER-positive	Somatotype at age 7	Large (5-9)	0.73	(0.50-1.04)	0.75	(0.52-1.08)	negative	0.09
2010	Jingmei Li	100/862	postmenopausal, ER-negative	Somatotype at age 7	Lean (1-2)	1.00	Referent	1.00	Referent	~	~
2010	Jingmei Li	34/428	postmenopausal, ER-negative	Somatotype at age 7	Medium (3-4)	0.66	(0.43-0.99)	0.66	(0.44-1.01)	inconclusive	0.00
2010	Jingmei Li	5/108	postmenopausal, ER-negative	Somatotype at age 7	Large (5-9)	0.34	(0.14-0.87)	0.36	(0.14-0.90)	negative	0.05
2010	Jingmei Li	445/862	postmenopausal, PR-positive	Somatotype at age 7	Lean (1-2)	1.00	Referent	1.00	Referent	~	~
2010	Jingmei Li	170/428	postmenopausal, PR-positive	Somatotype at age 7	Medium (3-4)	0.77	(0.62-0.95)	0.77	(0.62-0.96)	inconclusive	0.00
2010	Jingmei Li	44/108	postmenopausal, PR-positive	Somatotype at age 7	Large (5-9)	0.73	(0.50-1.07)	0.76	(0.52-1.12)	negative	0.13
2010	Jingmei Li	155/862	postmenopausal, PR-negative	Somatotype at age 7	Lean (1-2)	1.00	Referent	1.00	Referent	~	~
2010	Jingmei Li	59/428	postmenopausal, PR-negative	Somatotype at age 7	Medium (3-4)	0.77	(0.55-1.06)	0.77	(0.56-1.07)	inconclusive	0.00
2010	Jingmei Li	9/108	postmenopausal, PR-negative	Somatotype at age 7	Large (5-9)	0.43	(0.21-0.87)	0.44	(0.21-0.89)	negative	0.03
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Childhood somatotype	Per 1-unit increase	0.93	(0.86,1.01)	0.98	(0.90,1.07)	negative	71 %
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Childhood somatotype	Per 1-unit increase	0.89	(0.83,0.96)	0.92	(0.85,0.99)	negative	26 %**

Table 2.4c. Changes in the OR/RRs of childhood and adolescent body fatness by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Adolescent somatotype	Per 1-unit increase	0.90	(0.82,0.99)	0.97	0.88,1.07)	negative	73 %*
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Adolescent somatotype	Per 1-unit increase	0.86	(0.80,0.93)	0.90	(0.83,0.97)	negative	26 %**
2011	Harris	456/725	All subjects	Body Fatness (ages 5–10)	1	1.00	Referent	1.00	Referent	~	~
2011	Harris	433/758	All subjects	Body Fatness (ages 5–10)	1.5–2	0.92	(0.77-1.09)	0.91	(0.77-1.08)	inconclusive	-0.13
2011	Harris	294/564	All subjects	Body Fatness (ages 5–10)	2.5–3	0.85	(0.70-1.02)	0.89	(0.74-1.08)	negative	0.28
2011	Harris	183/416	All subjects	Body Fatness (ages 5–10)	3.5–4	0.73	(0.59-0.90)	0.83	(0.66-1.03)	negative	0.41
2011	Harris	119/290	All subjects	Body Fatness (ages 5–10)	≥4.5	0.67	(0.52-0.86)	0.77	(0.60-0.99)	negative	0.35
2011	Harris	456/725	All subjects	Body Fatness (ages 11–20)	1	1.00	Referent	1.00	Referent	~	~
2011	Harris	433/758	All subjects	Body Fatness (ages 11–20)	1.5–2	0.85	(0.67-1.07)	0.84	(0.66-1.06)	inconclusive	-0.07
2011	Harris	294/564	All subjects	Body Fatness (ages 11–20)	2.5–3	0.79	(0.62-0.99)	0.82	(0.64-1.03)	negative	0.16
2011	Harris	183/416	All subjects	Body Fatness (ages 11–20)	3.5–4	0.73	(0.57-0.94)	0.82	(0.64-1.06)	negative	0.37
2011	Harris	119/290	All subjects	Body Fatness (ages 11–20)	≥4.5	0.58	(0.44-0.78)	0.71	(0.53-0.95)	negative	0.37

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated;
PE was estimated using the following formula: PE = $[\beta_{(unadjusted)}-\beta_{(adjusted)}]/\beta_{(unadjusted)}$
PM was reported in the paper, denoted as a percentage.
* $p < 0.05$, ** $p < 0.01$

Table 2.5. Changes in the OR/RRs of reproductive factors by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at menarche, ≥14	1.00	Referent	1.00	Referent	~	~
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at menarche, 12-13	0.98	(0.83-1.15)	1.01	(0.86-1.19)	inconclusive	1.49
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at menarche, <12	0.96	(0.69-1.33)	1.02	(0.73-1.41)	inconclusive	1.49
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Age at menarche Per 2-year increase	0.85	(0.74-0.98)	0.83	(0.72-0.96)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Age at menarche Per 2-year increase	0.92	(0.80,1.05)	0.93	(0.81,1.06)	negative	11 %
2018	Rice	573/2018	NHS/NHSII, premenopausal at mammogram	Age at menarche Per 2-year increase	0.86	(0.75,1.00)	0.84	(0.73,0.98)	inconclusive	NM
2018	Rice	1197/3002	NHS/NHSII, postmenopausal at mammogram	Age at menarche Per 2-year increase	0.96	(0.87,1.07)	0.95	(0.85,1.06)	inconclusive	NM
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Nulliparous vs parous	1.15	(0.86,1.52)	1.07	(0.80,1.42)	positive	52 %
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Nulliparous vs parous	1.22	(0.88,1.69)	1.12	(0.80,1.56)	positive	43 %
2018	Rice	1095/3180	NHS/NHSII, premenopausal at mammogram	Nulliparous vs parous	1.14	(0.96,1.35)	1.08	(0.91,1.29)	positive	40 %
2018	Rice	2158/5575	NHS/NHSII, postmenopausal at mammogram	Nulliparous vs parous	1.23	(1.07,1.41)	1.13	(0.98,1.29)	positive	43 %**
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Parity (among parous) Per one-child increase	1.00	(0.90,1.12)	1.03	(0.92,1.16)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Parity (among parous) Per one-child increase	1.02	(0.95,1.09)	1.03	(0.96,1.11)	inconclusive	NM
2018	Rice	499/1697	NHS/NHSII, premenopausal at mammogram	Parity (among parous) Per one-child increase	0.98	(0.87,1.09)	1.00	(0.89,1.12)	inconclusive	NM
2018	Rice	1028/2713	NHS/NHSII, postmenopausal at mammogram	Parity (among parous) Per one-child increase	0.99	(0.93,1.06)	1.01	(0.95,1.08)	inconclusive	NM
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Age at first birth (among parous) Per 5-year increase	1.18	(1.03,1.36)	1.18	(1.02,1.36)	positive	3%
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Age at first birth (among parous)	1.23	(1.07,1.41)	1.19	(1.04,1.38)	positive	13%*

Table 2.5. Changes in the OR/RRs of reproductive factors by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
				Per 5-year increase						
2018	Rice	802/2480	NHS/NHSII, premenopausal at mammogram	Age at first birth ≥ 30 versus < 30	1.32	(1.09,1.60)	1.30	(1.08,1.58)	positive	5%
2018	Rice	1727/4619	NHS/NHSII, postmenopausal at mammogram	Age at first birth ≥ 30 versus < 30	1.26	(1.08,1.47)	1.21	(1.03,1.42)	positive	16%
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Breastfeeding (among parous) Ever vs never	0.99	(0.76,1.28)	1.00	(0.77,1.30)	negative	89%
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Breastfeeding (among parous) Ever vs never	0.96	(0.79,1.16)	0.97	(0.80,1.17)	negative	22%
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Breastfeeding (among parous who ever breastfed) Per 12-month increase	0.95	(0.81,1.11)	0.93	(0.80,1.08)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Breastfeeding (among parous who ever breastfed) Per 12-month increase	1.25	(1.06,1.46)	1.25	(1.06,1.47)	inconclusive	NM
2016	Rice	559/1727	NHS/NHSII, premenopausal at mammogram	Birth index Per 102-unit increase	0.66	(0.43,1.01)	0.77	(0.50,1.19)	negative	38%
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Birth index Per 102-unit increase	0.96	(0.73,1.25)	1.04	(0.79,1.36)	inconclusive	NM
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Age at menopause Per 4-year increase	1.12	(1.05,1.20)	1.12	(1.04,1.19)	positive	5%
2018	Rice	1948/4646	NHS/NHSII, postmenopausal at mammogram	Age at menopause Per category increase	1.07	(1.02,1.13)	1.07	(1.02,1.13)	positive	1%
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at 1st birth, # of 1st degree relatives: <20 yrs, n=0	1.00	Referent	1.00	Referent	~	~
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at 1st birth, # of 1st degree relatives: 20-24 yrs, n=0	1.27	(1.09-1.48)	1.22	(1.05-1.42)	positive	0.17
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at 1st birth, # of 1st degree relatives: 25-29 yrs, n=0	1.62	(1.20-2.18)	1.50	(1.10-2.03)	positive	0.16
2005	Tice	81,777	All subjects, diverse racial/ethnic groups	Age at 1st birth, # of 1st degree relatives: 30+ yrs, n=0	2.05	(1.31-3.22)	1.83	(1.16-2.89)	positive	0.16

Table 2.5. Changes in the OR/RRs of reproductive factors by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	Age at first birth (parous), <26 years	1.00	Referent	1.00	Referent	~	~
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	Age at first birth (parous), ≥ 26	1.08	(0.93-1.26)	1.05	NA	positive	0.37
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	No. of children, 0	1.00	Referent	1.00	Referent	~	~
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	No. of children, 1–2	0.76	(0.62–0.93)	0.79	NA	negative	0.14
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	No. of children, ≥ 3	0.65	(0.53–0.80)	0.70	NA	negative	0.17
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	No. of children, age at first birth, Nulliparous	1.00	Referent	1.00	Referent	~	~
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	1–2 children, AFB < 26 yr	0.74	(0.59-0.93)	0.78	NA	negative	0.17
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	1–2 children, AFB ≥ 26 yr	0.77	(0.62-0.97)	0.80	NA	negative	0.15
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	≥ 3 children, AFB < 26 yr	0.66	(0.53-0.81)	0.71	NA	negative	0.18
2012	Woolcott	1699/2422	All subjects, 74% postmenopausal, pooled Ethnic groups	≥ 3 children, AFB ≥ 26 yr	0.64	(0.48-0.85)	0.69	NA	negative	0.17
2014	Fejerman	304/809	All subjects (Mexican)	Parity	0.87	(0.81-0.93)	0.88	(0.82-0.95)	negative	0.12
2014	Fejerman	304/809	All subjects (Mexican)	Breast feeding	0.76	(0.53-1.12)	0.77	(0.53-1.13)	negative	0.04

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated; NA: not available; NM: not mediated

PE was estimated using the following formula: PE = $[\beta_{(unadjusted)}-\beta_{(adjusted)}]/\beta_{(unadjusted)}$

PM was reported in the paper, denoted as a percentage.

* $p < 0.05$, ** $p < 0.01$

Table 2.6. Changes in the OR/RRs of exogenous hormone use and circulating sex hormone or antioxidants by adjustment for mammographic density

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2006	Boyld	107/112	NBSS (n = 416)	Hormone use, Never	1.00	Referent	1.00	Referent	~	~
2006	Boyld	52/59	NBSS (n = 416)	Hormone use, Past	0.99	(0.61-1.61)	1.04	(0.64-1.69)	inconclusive	4.90
2006	Boyld	45/41	NBSS (n = 416)	Hormone use, Current	1.13	(0.68-1.88)	1.12	(0.66-1.87)	positive	0.07
2006	Boyld	190/215	OBSP (n = 708)	Hormone use, Never	1.00	Referent	1.00	Referent	~	~
2006	Boyld	57/44	OBSP (n = 708)	Hormone use, Past	1.48	(0.95-2.32)	1.47	(0.93-2.32)	positive	0.02
2006	Boyld	103/99	OBSP (n = 708)	Hormone use, Current	1.20	(0.85-1.71)	1.12	(0.78-1.60)	positive	0.38
2006	Boyld	171/191	SMPBC (n = 617)	Hormone use, Never	1.00	Referent	1.00	Referent	~	~
2006	Boyld	65/52	SMPBC (n = 617)	Hormone use, Past	1.43	(0.93-2.22)	1.39	(0.90-2.16)	positive	0.08
2006	Boyld	75/63	SMPBC (n = 617)	Hormone use, Current	1.50	(0.99-2.27)	1.44	(0.95-2.18)	positive	0.10
2006	Boyld	468/518	Combined (n = 1,741)	Hormone use, Never	1.00	Referent	1.00	Referent	~	~
2006	Boyld	174/155	Combined (n = 1,741)	Hormone use, Past	1.27	(0.98-1.64)	1.27	(0.98-1.65)	inconclusive	0.00
2006	Boyld	223/203	Combined (n = 1,741)	Hormone use, Current	1.26	(1.00-1.59)	1.19	(0.94-1.51)	positive	0.25
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Hormone replacement therapy use Past vs never	1.18	(0.91,1.53)	1.12	(0.86,1.46)	positive	31%
2016	Rice	731/1695	NHS/NHSII, postmenopausal at mammogram	Hormone replacement therapy use Current vs never	1.71	(1.37,2.13)	1.52	(1.22,1.90)	positive	22%**
2018	Rice	1993/5083	NHS/NHSII, postmenopausal at mammogram	Hormone replacement therapy use Current vs never/former	1.39	(1.24,1.55)	1.23	(1.10,1.37)	positive	37% **
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Hormone use, Never	1.00	Referent	1.00	Referent	~	~
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Hormone use, Ever	1.56	(1.19-2.04)	1.49	(1.13-1.95)	positive	11% (4–30%)
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Hormone use, Current	1.87	(1.40-2.48)	1.76	(1.32-2.34)	positive	10% (4-22%)*
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Hormone use, Never	1.00	Referent	1.00	Referent	~	~
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Estrogen	0.99	(0.59-1.65)	0.94	(0.56-1.57)	inconclusive	-5.16
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Sequential estrogen/progestin	2.09	(1.48-2.97)	1.94	(1.37-2.69)	positive	0.10
2018	Azam	299/4272	DCH (n = 4501), postmenopausal	Continuous estrogen/progestin	3.39	(2.20-5.22)	3.21	(2.08-4.94)	positive	0.04

Table 2.6. Changes in the OR/RRs of exogenous hormone use and circulating sex hormone or antioxidants by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2018	Rice	1447/4077	NHS/NHSII, postmenopausal at mammogram	Hormone replacement therapy use Current E vs never/former	1.16	(1.00,1.34)	1.05	(0.90,1.22)	positive	69%
2018	Rice	1628/4076	NHS/NHSII, postmenopausal at mammogram	Hormone replacement therapy use Current E+P vs never/former	1.66	(1.45,1.90)	1.46	(1.27,1.67)	positive	26%**
2014	Schoemaker	265/343	postmenopausal	Oestradiol Q1	1.00	Referent	1.00	Referent	~	~
2014	Schoemaker	265/343	postmenopausal	Oestradiol Q2	1.18	(0.67-2.09)	1.21	(0.68-2.16)	inconclusive	-0.15
2014	Schoemaker	265/343	postmenopausal	Oestradiol Q3	1.51	(0.83-2.75)	1.49	(0.81-2.75)	positive	0.03
2014	Schoemaker	265/343	postmenopausal	Oestradiol Q4	2.07	(1.11-3.84)	2.03	(1.08-3.81)	positive	0.03
2014	Schoemaker	265/343	postmenopausal	Free oestradiol Q1	1.00	Referent	1.00	Referent	~	~
2014	Schoemaker	265/343	postmenopausal	Free oestradiol Q2	1.32	(0.74-2.36)	1.46	(0.81-2.63)	inconclusive	-0.36
2014	Schoemaker	265/343	postmenopausal	Free oestradiol Q3	1.72	(0.95-3.13)	1.78	(0.97-3.27)	inconclusive	-0.06
2014	Schoemaker	265/343	postmenopausal	Free oestradiol Q4	2.42	(1.27-4.61)	2.48	(1.29-4.78)	inconclusive	-0.03
2014	Schoemaker	265/343	postmenopausal	Testosterone Q1	1.00	Referent	1.00	Referent	~	~
2014	Schoemaker	265/343	postmenopausal	Testosterone Q2	1.34	(0.75-2.37)	1.21	(0.67-2.18)	positive	0.35
2014	Schoemaker	265/343	postmenopausal	Testosterone Q3	1.42	(0.79-2.52)	1.36	(0.76-2.44)	positive	0.12
2014	Schoemaker	265/343	postmenopausal	Testosterone Q4	2.11	(1.20-3.70)	2.01	(1.14-3.54)	positive	0.07
2014	Schoemaker	265/343	postmenopausal	Free testosterone Q1	1.00	Referent	1.00	Referent	~	~
2014	Schoemaker	265/343	postmenopausal	Free testosterone Q2	1.47	(0.82-2.64)	1.53	(0.85-2.76)	inconclusive	-0.10
2014	Schoemaker	265/343	postmenopausal	Free testosterone Q3	1.82	(1.01-3.28)	1.87	(1.03-3.40)	inconclusive	-0.05
2014	Schoemaker	265/343	postmenopausal	Free testosterone Q4	2.07	(1.15-3.74)	2.15	(1.18-3.91)	inconclusive	-0.05
2014	Schoemaker	265/343	postmenopausal	SHBG Q1	1.00	Referent	1.00	Referent	~	~
2014	Schoemaker	265/343	postmenopausal	SHBG Q2	1.10	(0.63-1.90)	1.11	(0.63-1.95)	inconclusive	-0.09
2014	Schoemaker	265/343	postmenopausal	SHBG Q3	0.86	(0.48-1.56)	0.74	(0.40-1.37)	inconclusive	-1.00
2014	Schoemaker	265/343	postmenopausal	SHBG Q4	0.64	(0.35-1.20)	0.58	(0.31-1.10)	inconclusive	-0.22
2007	Tamimi	253/520	postmenopausal	Plasma Estradiol Q1	1.00	Referent	1.00	Referent	~	~

Table 2.6. Changes in the OR/RRs of exogenous hormone use and circulating sex hormone or antioxidants by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2007	Tamimi	253/520	postmenopausal	Plasma Estradiol Q2	1.20	(0.80-2.00)	1.20	(0.80-2.00)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Plasma Estradiol Q3	1.20	(0.80-2.00)	1.30	(0.80-2.10)	inconclusive	-0.44
2007	Tamimi	253/520	postmenopausal	Plasma Estradiol Q4 adj. for Current BMI	2.40	(1.40-3.90)	2.40	(1.40-4.00)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Plasma Estradiol Q4 adj. for BMI at age 18	2.40	(1.50-3.80)	2.90	(1.80-4.60)	inconclusive	-0.22
2007	Tamimi	253/520	postmenopausal	Free Estradiol Q1	1.00	Referent	1.00	Referent	~	~
2007	Tamimi	253/520	postmenopausal	Free Estradiol Q2	1.10	(0.60-1.70)	1.10	(0.70-1.90)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Free Estradiol Q3	1.30	(0.70-2.10)	1.40	(0.80-2.40)	inconclusive	-0.28
2007	Tamimi	253/520	postmenopausal	Free Estradiol Q4	2.20	(1.30-3.70)	2.30	(1.30-4.00)	inconclusive	-0.06
2007	Tamimi	253/520	postmenopausal	Testosterone Q1	1.00	Referent	1.00	Referent	~	~
2007	Tamimi	253/520	postmenopausal	Testosterone Q2	0.80	(0.50-1.30)	0.80	(0.50-1.40)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Testosterone Q3	1.40	(0.90-2.20)	1.40	(0.90-2.30)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Testosterone Q4	1.80	(1.20-2.90)	2.00	(1.20-3.10)	inconclusive	-0.18
2007	Tamimi	253/520	postmenopausal	Free testosterone Q1	1.00	Referent	1.00	Referent	~	~
2007	Tamimi	253/520	postmenopausal	Free testosterone Q2	1.50	(0.90-2.50)	1.50	(0.90-2.50)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Free testosterone Q3	1.70	(1.00-2.80)	1.70	(1.00-2.80)	inconclusive	0.00
2007	Tamimi	253/520	postmenopausal	Free testosterone Q4	2.20	(1.30-3.60)	2.20	(1.30-3.80)	inconclusive	0.00
2009	Tamimi	604/626	postmenopausal	α-Carotene Quin1	1.00	Referent	1.00	Referent	~	~
2009	Tamimi	604/626	postmenopausal	α-Carotene Quin2	1.10	(0.80-1.60)	1.10	(0.70-1.60)	inconclusive	0.00
2009	Tamimi	604/626	postmenopausal	α-Carotene Quin3	1.10	(0.80-1.60)	1.00	(0.70-1.50)	positive	1.00
2009	Tamimi	604/626	postmenopausal	α-Carotene Quin4	0.80	(0.60-1.20)	0.70	(0.50-1.10)	inconclusive	-0.60
2009	Tamimi	604/626	postmenopausal	α-Carotene Quin5	0.70	(0.40-1.00)	0.60	(0.40-0.90)	inconclusive	-0.43
2009	Tamimi	604/626	postmenopausal	β-Carotene Quin1	1.00	Referent	1.00	Referent	~	~
2009	Tamimi	604/626	postmenopausal	β-Carotene Quin2	1.30	(0.90-1.80)	1.30	(0.90-1.80)	inconclusive	0.00
2009	Tamimi	604/626	postmenopausal	β-Carotene Quin3	1.40	(1.00-2.10)	1.30	(0.90-2.00)	positive	0.22

Table 2.6. Changes in the OR/RRs of exogenous hormone use and circulating sex hormone or antioxidants by adjustment for mammographic density (cont.)

Year	First Author	No. subjects	Subjects	Exposure	Unadj. OR/RR	95% CI	Adj. OR/RR	95% CI	Sign of NIE	PE Index
2009	Tamimi	604/626	postmenopausal	β-Carotene Quin4	0.90	(0.60-1.40)	0.90	(0.60-1.30)	inconclusive	0.00
2009	Tamimi	604/626	postmenopausal	β-Carotene Quin5	0.60	(0.40-1.00)	0.60	(0.40-0.90)	inconclusive	0.00
2009	Tamimi	604/626	postmenopausal	Total carotenoids Quin1	1.00	Referent	1.00	Referent	~	~
2009	Tamimi	604/626	postmenopausal	Total carotenoids Quin2	1.10	(0.80-1.50)	1.00	(0.70-1.50)	positive	1.00
2009	Tamimi	604/626	postmenopausal	Total carotenoids Quin3	1.20	(0.80-1.70)	1.10	(0.70-1.60)	positive	0.48
2009	Tamimi	604/626	postmenopausal	Total carotenoids Quin4	0.80	(0.50-1.10)	0.70	(0.50-1.00)	inconclusive	-0.60
2009	Tamimi	604/626	postmenopausal	Total carotenoids Quin5	0.70	(0.50-1.00)	0.60	(0.40-0.90)	inconclusive	-0.43

Unadj.: Unadjusted; Adj.: adjusted; NIE (natural indirect effect); PE: proportion explained; PM: proportion mediated; NA: not available; CMSP: Copenhagen mammography screening program; Q1: the first quartile; Quin1: the first quintile
PE was estimated using the following formula: PE = $[\beta_{(unadjusted)}-\beta_{(adjusted)}]/\beta_{(unadjusted)}$
PM was reported in the paper, denoted as a percentage.
* $p < 0.05$, ** $p < 0.01$

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CHAPTER 3: DATA AND METHODOLOGY

3.1 DATASETS AND VARIABLES

3.1.1 Seattle Data

The first data is from a combined breast cancer case-control study in which information on the extent of mammographic density and mammographic density patterns were ascertained from the results of previous mammograms. Additional details about the data set used are described in Thomas et al. [1].

Cases (n=547) and controls (n=472) were recruited from women who had participated in four previous population-based case-control studies of breast cancer in the Seattle area: BCYW [2]; WISH [3]; HORMONE [4]; and EMF [5]. Cases from all four studies were identified through the Cancer Surveillance System, a population-based cancer registry sponsored by the Surveillance, Epidemiology, and End Results program of the National Cancer Institute that covers 13 counties of Western Washington State [6]. Women with an initial diagnosis of either in situ or invasive disease were included in all four studies.

Cases eligible for the BCYW study were female residents of King, Pierce, on Snohomish counties who were born after 1944 and who developed breast cancer from January 1983 through April 1990. Cases eligible for the WISH study were those from the same three counties <45 years of age who were diagnosed from May 1990 through December 1992. The HORMONE study included the cases 50-64 years of age residing in King County, diagnosed from January 1988 to June 1990. Cases eligible for the EMF study were female residents of King and Snohomish counties diagnosed from January 1993 to June 1995. To eliminate women unlikely to have had access to mammographic screening before age 50 or to have had their mammograms in the too distant past for likely retrieval, cases diagnosed before 1985 in the BCYW studies, those >54 years of age in the HORMONE study, and those >59 years of age in the EMF study were not considered eligible for this investigation. Controls for all four studies were selected by random digit dialing, using a modification of the Waksberg method [7]. Controls were frequency matched to cases on age and county of residence.

Experienced interviewers had administered standardized questionnaires to all consenting study subjects after obtaining written informed consent. Although these questionnaires varied among the four prior studies, they were comparable on the standard risk factors for breast cancer which included information on marital, reproductive, menstrual and contraceptive history, use of exogenous hormones (oral contraceptives and estrogen hormone replacement therapy), lifestyle factors, prior breast biopsies, socioeconomic characteristics, and family history of breast cancer. Weight one year before the interview and the maximum height attained were also ascertained at the interview, except in the WISH study where height was measured; these data were used to calculate body mass index (weight in kilograms ÷ height in meters squared). Information on all potential breast cancer risk/protective factors from questionnaires and datasets of the different studies was combined in a systematic manner into the variables included in our dataset.

A history of prior mammographic screening was also elicited from the women. The women, or their next of kin if the woman was deceased, were sent a questionnaire to ascertain information on the time and place each screening mammogram was taken and a consent form giving their permission for us to contact the radiologist and request a loan of the mammograms was signed. Telephone calls were made to women who did not respond, and in some instances, the questionnaire was administered during the call.

The craniocaudal and mediolateral oblique or lateral radiographs were both used to classify each breast according to the parenchymal pattern classification of Wolfe [8, 9], including N1 [mostly fat (radiolucent), few ducts], P1 [ductal (linear) patterns and nodular densities occupying >25% of the area], and DY (dense sheets, no ductal pattern discernable). The reference radiologist, who classified the mammograms, received training from a colleague of Wolfe (Martine Salane) to enhance compatibility with prior investigations. In future analyses, women were categorized into having P2 and DY patterns ("high risk") and N1 and P1 patterns ("lower risk").

The radiologist traced the outline of the dense areas on the craniocaudal view with a wax (China) marker. A single technician, who had also received training from Dr. Wolfe's associate, then measured the areas of the breast and the dense area with a compensating polar planimeter (LASICO, Los Angeles, CA). In future analyses, women were categorized into the upper quartile

of percent breast density in controls ("high risk": ≥70.3%) versus all other women ("lower risk": ≤70.2%).

3.1.2 Mayo Data

The second dataset is from the Mayo Mammography Health Study (MMHS), which is a prospective cohort, comprised of 19,924 women (51.2% adjusted response rate) ages 35 and over, residing in the tri-state region surrounding the Mayo Clinic in Rochester, MN. (Minnesota, Iowa, and Wisconsin), without a personal history of breast cancer, who were scheduled for a screening mammogram at the Mayo Clinic between October 2003 and September 2006 [10]. All women had a 4-view screening mammogram at the time of enrollment and completed a self-administered questionnaire. A total of 2,284 women in the cohort reported having had at least one form of cancer (other than breast cancer) prior to enrollment. The investigators defined a healthy cohort as the 17,639 women who were free of a history of any cancer at baseline (excluding non-melanoma skin cancer).

Follow-up for cancer occurrence was performed annually by linking to Mayo Clinic databases and the tri-state cancer registries. Active follow-up for cancer and vital status was conducted via mail and telephone from women who had not been back to the Mayo Clinic within 12 months and either had moved out of the tri-state region or did not grant consent for registry linkage. Telephone follow-up was attempted on non-responders to the mailed contact. As of December 2013, the total number of incident cancers in the healthy cohort was 1601, of which 665 were breast cancers.

Percent mammographic density (dense area divided by total area, times 100%) was estimated using a computer-assisted thresholding program, Cumulus, on the enrollment screening mammogram from all participants in the case-cohort and nested case-control studies. Clinical BI-RADS four-category tissue composition assessments corresponding to the enrollment mammogram were obtained from the Mayo Clinic electronic medical record. The BI-RADS tissue composition has been routinely estimated on all screening mammograms at the Mayo Clinic since mid-1996. Mayo Clinic attending radiologists classified each mammogram into one of four

categories as defined in the BI-RADS lexicon over this period (American College of Radiology, third edition): (a) the breast is almost entirely fat; (b) there are scattered fibroglandular densities; (c) the breast tissue is heterogeneously dense, which may lower the sensitivity of mammography; and (d) the breast is extremely dense, which could obscure a lesion on mammography. These ratings convey the relative possibility that a lesion may be obscured in mammography. All four mammogram views (craniocaudal and mediolateral oblique for ipsilateral and contralateral sides) contribute to the assessment of BI-RADS composition. In our study, we used the estimates that experienced radiologists assessed in the clinical setting. These radiologists did not systematically assess BI-RADS composition for this study, but this rating has shown adequate inter-observer reliability

3.2 STUDY AIMS AND HYPOTHESIS

Previous analyses from these two datasets have shown associations between some breast cancer risk factors and mammographic density. We proposed to extend the analysis of these two data sets for the following three specific aims:

1) To identify the key risk/protection factors that meet the criteria 1 and 2 for a mediator/surrogate marker. That is, the exposures should be associated with mammographic density and mammographic density should predict the risk of breast cancer.
2) To determine whether mammographic density accounted for any part of the association between any of the risk/protection factors and breast cancer risk. These would include a measure of direct and indirect effect as well as a formal statistical test of the significance of the mediation effect.
3) To quantify the extent to which the observed association between the identified risk factors for breast cancer is mediated through mammographic density. These would include measures of mediation such as proportion mediated and test of significance as well as a 95% CI.

The primary purpose of this project was to determine whether mammographic density was in the causal pathway by which traditional breast cancer risk/protective factors are related to breast

cancer. We hypothesized that mammographic density at least partially mediated some of the known factors and breast cancer associations. We further hypothesized that some risk factors may affect pre-menopausal and postmenopausal women differentially.

3.3 METHODS

3.3.1 Theoretical Framework

The basic path diagram representation of the conceptual relationships between variables for the mediator analysis is illustrated in Figure 3.1 where: the coefficients represent the regression coefficients along the paths from the risk factors F to the mediator M (ex. BMI to mammographic density), b is the coefficient for the direct path from the mediator M to the outcome B (ex. mammographic density to breast cancer), the c coefficients represent the unmediated paths from the risk factors F to the outcome B (ex. BMI to cancer), and d represents the coefficient for different confounding covariates C in the model [11].

3.3.2 Selection of Variables

Two important criteria must be satisfied for a mediator: the exposures should be associated with breast density and breast density should predict the risk of breast cancer. Statistical analysis proceeded by first narrowing the set of risk factors to be those that were associated with breast density, using requirement 1 stated in the introduction, so as to identify which factors may act through breast density to increase or decrease the risk of breast cancer. Factors need not statistically predict the risk of breast cancer. But it would be good to check them for the direction of mediation.

Variables were selected based on the literature and further confirmed by regression analysis. First, the relationships between potential breast cancer risk factors and the development of breast cancer and measures of mammographic density in the data set were examined to determine which variables should be included in the statistical models. Logistic regression was used for case-control study and Cox proportional hazards regression was used for the case-cohort design. Attempts were made to determine if there were additional variables that confounded the potential risk/protective factor – breast cancer relationship by successively adding all other non-

identical potential confounders (other risk/protective factors) to the regression models and determining whether they changed the odds ratio by more than 10 percent. The same confounders were then added to the models investigating the relationship with these risk/protective factors with measures of mammographic density. We then selected variables to be used in future analyses if they had such a strong association with measures of mammographic density that they may indicate an important mediator effect in the path models. Preference was given if variables displayed a strong or significant relationship with breast cancer (or were a major confounder of such an association). Factors that did not predict breast cancer risk should also be considered because they may show inconsistent mediation. The Gail model and other risk assessment models incorporating breast density have been developed and could serve as references. The selection also depends on the availability of variables in the data set. Table 3.1 shows a list of potential risk factors of breast cancer that were likely to act through the breast density pathway.

3.3.3 Model Specification and Statistical Analysis

VanderWeele and colleagues have outlined the analytic approaches that are available to conduct mediation analysis based on the counterfactual framework [12]. Let F denote a traditional risk factor, M the percentage mammographic density, B breast cancer, and C a set of baseline covariates. The F–B pathway is independent of breast density, whereas the F–M–B pathway describes that the effect of the risk factor is mediated through breast density. We could fit the following logistic regression models:

$$\text{logit}[P(B = 1|F, M, C)] = \theta_0 + \theta_1 F + \theta_2 M + \theta_3 FM + \theta_4 C$$

$$M = \beta_0 + \beta_1 F + \beta_2 C + \varepsilon$$

SAS and SPSS macros are available to do the above mediation analysis automatically (reference). It fits two regression models simultaneously: one viewing breast cancer as the outcome variable with breast density as a covariate, and the other viewing upper-quartile of breast density as the outcome variable.

The use of mediator models allowed us to determine the extent to which breast cancer risk/protective factors acted through mammographic density (indirect effect) or around it (direct or unmediated effect) in increasing or decreasing the risk of breast cancer. However, it is very important to adequately control for covariates C, namely the exposure-outcome, mediator-outcome, and exposure-mediator confounders.

3.3.4 Sensitivity Analysis

It is impossible to include all the variables to the model, especially when the sample size is not large enough. We can only choose the most significant ones. In this case, sensitivity analysis is important in assessing the extent to which uncontrolled confounding may or may not substantially influence estimates.

3.3.5 Comparison of Methods

Over the past decade, studies of causal mediation have grown rapidly in different fields. Several mediation analysis packages in different software (SAS, R, STATA, SPSS, etc.) have been developed. In our project, we focus on conducting causal mediation analysis following the approach outlined by VanderWeele and Vansteelandt [13], implemented using a SAS macro that can accommodate the case-control design [14]. For comparison purposes, we also conducted mediation analysis based on the "difference method" when the results with and without an exposure × PMD interaction were comparable. The mediation analysis using the "difference method" is outlined by Lin et al. [15], implemented using a SAS macro developed by Spiegelman and colleagues [16]. Other causal mediation analyses such as the R packages "mediation" and "medflex" are also considered. The latter is based on the class of natural effect models (NEMs) originally introduced by Lange et al. [11] and Vansterlandt et al. [12] and implemented in the R package medflex [2].

TABLE AND FIGURE

Table 3.1. List of potential risk factors and covariates from literature

	Risk factor (F) and Covariates (C)
Factors associated with breast density	**Age and Menopause**
	Reproductive Variables • Parity: nulliparous vs parous, later age at first birth, and fewer live births have been associated with greater risk of breast cancer and with a higher proportion of dense breast tissue.
	Bodyweight and height **Nutrition, alcohol, and exercise** **Family history, race**
	• Family history (breast, ovarian, endometrial, and colon) • Age, parity, age at first live birth, age at menarche • HRT, menopausal status, • BMI, height, waist-to-hip ratio, alcohol consumption, smoking, education • Tamoxifen • Breast characteristics (involution, presence of atypia on a breast biopsy)
Factors that have not been shown to be associated with breast density	• Oral contraceptive use, Estradiol, oophorectomy, • Diet (low fat, polyunsaturated fat, vitamin C, E, B12, D supplement, folate) and physical activity (no consensus) • Aromatase inhibitors, raloxifene, aspirin

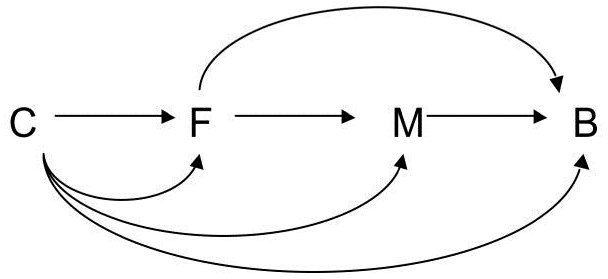

Figure 3.1. Conceptual relationships between variables illustrating mediation. Conceptual relationships illustrating mediation between a traditional breast cancer risk factor (F), mammographic density (M), and breast cancer risk (B), along with baseline confounding covariates (C).

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CHAPTER 4: STUDY 1

A Mediation Analysis of the Pooled Data from Four Population-Based Case-Control Studies in the Seattle Region

Abstract

Purpose: We conducted a causal mediation to examine whether and to what extent percent mammographic density (PMD) is in the pathway by which various breast cancer risk factors influence the risk of breast cancer.

Methods: Data were pooled from four population-based case-control studies conducted in the western Washington state, containing 547 breast cancer cases and 472 controls who had screening mammograms under age 50. We estimated the direct effects of various risk factors on risk of breast cancer and their indirect effects (i.e. effects mediated through PMD), as well as the proportion mediated by PMD.

Results: The association between breast calcifications and risk of breast cancer was partially mediated by PMD (proportion mediated = 29.0%), with an indirect-effect odds ratio of 1.16 (95% CI: 1.04-1.30; P = 0.009). PMD mediated 48.6% of the reduced risk among parous versus nulliparous women, which yielded an indirect-effect odds ratio of 0.76 (95% CI: 0.65-0.90; P = 0.001). No significant mediation by PMD was observed with respect to a first-degree family history of breast cancer, age at first live birth, and smoking. There was inconsistent mediation for the effect of adult body mass index.

Conclusions: Densities in mammograms from women <50 years of age partially mediated the effects of breast calcifications and being parous on the risk of breast cancer but appeared not to mediate the influence of a first-degree family history of breast cancer or age at first live birth. Some risk factors for breast cancer may alter risk partially by increasing mammographic densities.

4.1 INTRODUCTION

Several independent observations support the hypothesis that some established breast cancer risk factors may be mediated by their intermediate effects on the mammary tissue, which is evaluated by mammographic densities. First, mammographic breast density has been repeatedly shown to be one of the strongest independent risk factors for breast cancer [1-4]. Second, mammographic density has also been shown to be associated with a wide array of risk factors for breast cancer including age, menopausal status, age at first live birth, parity, body mass index (BMI), physical activity, alcohol consumption, hormone replacement therapy, endogenous levels of insulin-like growth factor 1 (IGF1) and prolactin, and family history of breast cancer [1]. Furthermore, breast density can even be changed by several exposures or interventions that are also known to influence breast cancer risk [5]. For example, tamoxifen, an anti-estrogen, was reported to reduce mammographic breast density as well as the risk of breast cancer [6-9].

Biologically, mammographic breast density has the potential to act as a mediator for breast cancer risk. Fatty tissue in the breast is relatively transparent to x-rays and appears dark on mammograms. Fibroglandular tissue, which consists of epithelial cells that line the ducts and their supporting fibrous connective tissue, is more radiologically dense and appears light on mammograms. Histological assessment of the dense and non-dense areas of the breast revealed that the dense tissue has a greater amount of epithelium and stroma, particularly collagen, increased nuclear occupation, lesser fat, and a higher proportion of proliferative disease without atypia than the non-dense breast tissue [10-12]. The proportion of a mammogram that is dense is thus an indirect measure of the amount of epithelial tissue in the breast. Since this is the tissue from which mammary carcinomas arise, the greater the percent mammographic density (PMD), the greater the number of cells available for malignant transformation. The increase in PMD may also reflect alteration of the stromal architecture and composition of the extracellular matrix such as collagen, which is a well-recognized component of both benign and malignant breast pathologies [13-15], In addition, the epithelium and stromal tissues may be a site for local inflammation, which could increase the risk of breast cancer [15]. Breast dense tissue has showed decreased alternatively activated macrophages in the stroma [15]. Both case-control studies and prospective studies have shown an increased risk of subsequent cancer to be correlated with PMD [4, 16, 17]. A risk factor for breast cancer could theoretically increase risk by altering the number of epithelial cells at risk

of a malignant transformation (as measured by the PMD), in which case we would observe that the risk factor appears to be mediated by its effect on the observed PMD [18]. Alternatively, a risk factor could be independent of any effect on the number of epithelial cells, and there would not be evidence for mediation by PMD.

Few studies have attempted to assess the role of mammographic density as a mediator for breast cancer risk, and the extent of mediation is rarely quantified using statistical mediation analyses. Seven studies have calculated the percent change in the odds ratios (ORs) and/or the "proportion explained (PE)" index [19-25], a measure of mediation by the traditional "difference method" comparing regression coefficients between models with and without the mediator [26]. Of these, ninety-five percent confidence intervals or p-values for the PE were available in only four studies [19, 22-24]. The "difference method" has been criticized for lacking a causal interpretation [27] and may provide biased estimators regarding mediation, especially for binary outcomes [28]. To address some of these limitations, the counterfactual approach has been proposed [29-32]. This approach emphasizes assumptions regarding confounding required for causal interpretation, with a formal definition of direct and indirect effects in a counterfactual framework, which allows for the decomposition of a total effect into direct and indirect effects, even in models with interactions and nonlinearities. This approach has recently been modified for use in analyzing data from case-control studies [32]. To date, only one study has attempted to use causal mediation analysis to evaluate the effect of a number of known risk factors on breast cancer through mammographic density [22].

Thus, the main purpose of this study was to estimate, for each breast cancer risk factor of interest, how much of its effect on risk is due to its influence on mammographic densities (its indirect effect) and how much of its effect on risk is a direct effect (not mediated through mammographic density). To do this, we used the analytic approaches based on the counterfactual framework described by Vanderweele et. al. [33] using the combined data from four population-based case-control studies [34].

4.2 METHODS

Study Design and Participants

The data source, study design, and participant characteristics have been described in detail previously [34]. Briefly, cases (n = 547) and controls (n = 472) were recruited from women who had participated in four previous population-based case-control studies of breast cancer in the Seattle area: BCYW (Breast Cancer in Young Women) [35], WISH (Women's Interview Study of Health) [36], HORMONE (Hormone Replacement Therapy and Breast Cancer in Middle-Aged Women) [37], and EMF (Electric Power and Risk of Breast Cancer) [38]. Women with an initial diagnosis of either in situ or invasive disease were included in all four studies. Controls for all four studies were selected by random digit dialing, using a modification of the Waksberg method [39]. Controls were frequency matched to cases on age and county of residence. This study has been approved by the Institutional Review Boards of the University of Illinois at Urbana Champaign and the Fred Hutchinson Cancer Research Center.

Measurement of Mammographic Density and Calcification

The earliest mammogram available on each study participant was obtained from local mammography facilities. Both craniocaudal and mediolateral oblique or lateral radiographs were used. A single reference radiologist traced the outline of the dense areas on the craniocaudal view with a wax (China) marker. A single technician then measured the areas of the breast and the dense area with a compensating polar planimeter (LASICO, Los Angeles, CA). PMD was calculated as the percentage of the area of the mammogram that was mammographically dense. The mean value of the percent density of both breasts was used.

The two views were also used to record the morphological type and distribution of all calcifications. This information was subsequently used to classify all mammographic calcifications on a scale of 1–5 in descending order of suspicion for existing carcinoma. The system used combined entities in the Breast Imaging Reporting and Data System (BI-RADS) of the American College of Radiology [40]. The BI-RADS designation of amorphous calcifications was not used.

Breast cancer risk factors and confounders

Experienced interviewers had administered standardized questionnaires to all consenting study subjects after obtaining written informed consent. Although these questionnaires varied among the four prior studies, they were comparable on the standard risk factors for breast cancer which included information on marital, reproductive, menstrual and contraceptive history, use of exogenous hormones (oral contraceptives and postmenopausal hormone replacement therapy), lifestyle factors, prior breast biopsies (including, but not distinguishing, needle aspiration, a biopsy of a lesion, and lumpectomy), socioeconomic characteristics, and family history of breast cancer. Weight at 1 year before the interview, weight at 18 years of age, and maximum height attained were also ascertained at the interview, except in the WISH study, in which height was measured; and these data were used to calculate the BMI.

Statistical Methods

We conducted a causal mediation analysis following the approach outlined by VanderWeele and Vansteelandt [32], implemented using a SAS macro that can accommodate the case-control design [41]. The analysis proceeded by first fitting an unconditional logistic regression model for breast cancer on the potential risk factor and PMD, adjusting for matching variables (study and age) and the baseline covariates. Second, a linear regression was fit for PMD on the potential risk factor and covariates using the controls. The regression for PMD and the regression for breast cancer risk were combined to obtain the ORs and 95% CIs for the following effects: (a) the natural direct effect (NDE) (i.e., the effect of the exposure on breast cancer risk not through PMD if PMD was fixed at the level that it would have been without the exposure), (b) the natural indirect effect (NIE) (i.e., the effect of the exposure on breast cancer risk through PMD), and (c) the total association between the exposure and breast cancer risk. Despite a lack of statistical significance on all exposure–PMD interaction terms, we compared the results with and without an exposure × PMD interaction term. We used both the delta method and bootstrap sampling (1000 samples) to obtain 95% confidence intervals (CIs). The proportion mediated was estimated by the equation of $OR^{NDE} \times (OR^{NIE} -1)/(OR^{NDE} \times OR^{NIE} -1)$, where OR^{NDE} is the direct-effect odds ratio and OR^{NIE} is the indirect-effect odds ratio [32]. All the p-values used were two-

sided. Type I errors were set at 0.10. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina, USA).

Sensitivity Analysis

To assess the robustness of our results, we repeated the analyses by restricting the data to premenopausal women at mammography date. Analyses were also performed by excluding women who had stopped bleeding at least four years by mammography date and similar results were obtained (not shown). Since the results with and without an exposure × PMD interaction were comparable, we also conducted a mediation analysis using the "difference method" outlined by Lin et al. [42], implemented using the SAS macro developed by Spiegelman and colleagues [43]. To determine the causal direction of the relationship between mammographic density and the risk factors of calcification and breast biopsy history, we also conducted mediation analyses using these two risk factors as potential mediators for the effect of PMD by assuming the mediation path in a way that PMD preceded these two risk factors.

4.3 RESULTS

The characteristics of the study population and the set of potential risk factors and selected confounders are shown in Table 4.1. We identified a total of 547 cases and 472 controls who had a screening mammogram before 50 years of age and 1 year or more prior to the date of diagnosis (for the cases) or reference date (for the controls). The participants were largely white women (97%) and less than 5% were aged 55 or older. More than 70% of women had their screening mammograms between the age of 35 and 45 years old.

Having had calcifications (OR = 1.263; 95% CI: 1.164, 1.371), first degree family history of breast cancer (OR = 2.066; 95% CI: 1.456, 2.960), ever having had a live birth (OR = 0.671; 95% CI: 0.480, 0.935) and per year increase in age at first live birth among parous women (OR = 1.042, 95% CI: 1.010, 1.075) were significantly associated with risk of breast cancer (Table 4.2). BMI at 1 year before the interview, BMI at 18 years old, history of breast biopsy/aspiration/lumpectomy, smoking, and the number of live births (among parous women)

were not significantly associated with breast cancer risk. Mediation analyses using the "difference method" showed that a first-degree family history of breast cancer, smoking, and age at the first live birth were not mediated by PMD, whereas having had calcifications and ever having had a live birth were partially mediated by PMD (PE = 16.5% and 42.5% respectively, p = 0.0001 for both).

The results of the causal mediation analyses for the whole data set were shown in Table 4.3 and Supplementary Table 4.1. Having had calcifications was directly associated with breast cancer risk (OR_{NDE}, 1.66; 95% CI, 1.26-2.19; P < 0.001), but it was also indirectly associated with breast cancer risk through its effect on mammographic density (OR_{NIE}, 1.16; 95% CI, 1.04-1.30; P = 0.009), with a proportion of 29.0% mediated by PMD. Although a first-degree family history of breast cancer was a significant breast cancer risk factor ($OR_{total\ effect}$, 1.893; 95% CI, 1.26858, 2.82474; P = 0.002), no significant mediation by PMD was observed (OR_{NIE} 0.891, 95% CI: 0.75642, 1.04965; P = 0.168). Ever having had a live birth exhibited a significant negative association with breast cancer ($OR_{total\ effect}$, 0.61; 95% CI, 0.42-0.89; P = 0.010), which was decomposed into a significant indirect-effect odds ratio of 0.76 (95% CI, 0.65-0.90; P = 0.001) through PMD and a direct-effect odds ratio of 0.80 (95% CI, 0.57-1.13; P = 0.207). Overall, 48.6% of the reduced risk of breast cancer related to being parous was attributable to lower PMD. Among parous women, per year increase in age at first live birth had a significant total effect ($OR_{total\ effect}$, 1.04; 95% CI, 1.00-1.07; P = 0.043) on the risk of breast cancer. However, the association was not mediated through PMD (OR_{NIE}, 0.999; 95% CI, 95% CI, 0.98-1.01; P = 0.88).

Although the BMI measures were not significantly associated with risk of breast cancer in our data, results from casual mediation analyses suggested the presence of inconsistent mediation or suppression, in which the direct and indirect effects had opposite directions while the total effect was not significant. BMI at 1 year before interview displayed a highly significant negative indirect association with breast cancer risk through PMD (OR_{NIE}, 0.940; 95% CI, 0.92-0.96; P=0.000), while it displayed a significant positive relationship with breast cancer risk (OR_{NDE}, 1.048; 95% CI, 1.02-1.08; P=0.003) independent of PMD. The opposing direct and indirect effects resulted in an overall non-significant total effect of BMI ($OR_{total\ effect}$, 0.98; 95% CI, 0.96-1.01; P = 0.274). Similarly, BMI at 18 years old was not associated with the risk of breast cancer ($OR_{total\ effect}$, 0.970;

95% CI, 0.92-1.02; P = 0.256). It had a significant negative indirect association with breast cancer risk through PMD (OR_{NIE}, 0.931; 95% CI, 0.91-0.96; P = 0.000). However, the direct association acting independently of PMD was not significant, although positive (OR_{NDE}, 1.04; 95% CI, 0.99-1.10; P = 0.121). This indicates that BMI at a younger age might act predominantly through its negative association through breast density, whereas BMI at an older age may also act positively through pathways independent of breast density.

At the 10% significance level, smoking ($OR_{total\ effect}$, 1.28; 95% CI, 0.96-1.71; P = 0.092) and history of breast biopsy/aspiration/lumpectomy ($OR_{total\ effect}$, 1.36; 95% CI, 0.96-1.93; P = 0.083) were associated with increased risk of breast cancer. PMD mediated the association with history of breast biopsy/aspiration/lumpectomy (OR_{NIE}, 1.28; 95% CI, 1.11-1.49; P = 0.001) but not smoking (OR_{NIE}, 1.02; 95% CI, 0.92-1.14; P = 0.676). The proportion mediated was estimated to be 83.1%, which is higher than the PE index (64.4%) from the difference method. Note that the PE has a wide range of 95% CI, indicating that the PE estimate is very imprecise.

Including versus excluding the exposure-mediator interaction terms did not change the estimates of the direct and indirect effects much (Table 4.3). When analyses were restricted to premenopausal women, results were similar to that seen for all women (Supplementary Table 4.2).

4.4 DISCUSSION

Our results showed that PMD partially mediated the association between breast calcifications and ever having had a live birth with the risk of breast cancer. The associations of other factors with breast cancer risk, including a family history of breast cancer, age at first live birth, and smoking, were not mediated by PMD. Furthermore, we observed the presence of inconsistent mediation for adult BMI.

Calcifications are tiny mineral deposits within the breast tissue, which may show up as high-density white spots on a mammogram. It is well known that certain calcifications on a mammogram, particularly if small, multiple, and clustered, are predictive of a future diagnosis of breast tumors [44]. A third of breast cancers show calcifications as the only mammographically

suspicious feature [45]. Our results showed that about 29% of the increased risk of breast cancer in relation to having breast calcifications was mediated through PMD, while it also increased breast cancer risk through pathways independent of PMD. However, since calcifications and PMD were measured using the same mammogram, we cannot disentangle the direction of causality between calcifications and PMD. To further test our hypothesis, we conducted a sensitivity analysis by assuming the mediation path in a way that PMD precedes calcifications and that the effect of PMD on the risk of breast cancer is mediated by calcifications. Results showed that adjustment for calcifications led to only a minimal attenuation in the association between PMD and breast cancer risk, with less than 10% mediated by calcification (Supplementary Table 4.3). The results provided no support for the hypothesis that calcifications mediated the association of PMD with breast cancer risk even if we assume calcification precedes mammographic density. Therefore, it is more likely that PMD mediated the association between having breast calcifications and the risk of breast cancer. Biologically, microcalcification is an important feature in breast lesions and early signs of breast cancer. Of the breast cancers detected on mammography due to calcifications, about two-thirds represent ductal carcinoma in situ and the remainder are invasive ductal carcinoma [45]. It is thought to be a result of abnormal calcium deposition and mineralization of necrotic debris that is caused by rapidly proliferating tumor cells that use up the blood supply, resulting in cell death and subsequently increased acidosis in the microenvironment [46]. However, given that it is difficult to determine the temporality between these two breast cancer risk factors, this result should be interpreted cautiously.

Our results showed that PMD mediated 48.6% of the association between ever having had a live birth and breast cancer risk, which is supported by two previous studies[22, 23]. Although the calculated proportion mediated by PMD in these studies was only significant among postmenopausal women [23], the estimates (40-52%) were very similar to our observation, regardless of menopausal status. A potential mechanism for this finding of mediation might be a reduction in mammographic density after pregnancy [47]. Studies have consistently shown that nulliparous women had a greater percent density than parous women [48-52], and there is a negative association between increasing parity and mammographic density [48-51, 53-55].

A family history of breast cancer in first-degree relatives is an established risk factor for breast cancer. In our study, the increased risk of breast cancer in relation to a first-degree family history of breast cancer was found to be independent of PMD. This result of no mediation is consistent with two recent studies which showed that the association with a family history of breast cancer was not mediated by PMD in both premenopausal and postmenopausal women [22, 23]. However, in another study, PMD was found to explain 14% (95% CI, 4-39%) of the association of family history (at least one affected first-degree relative) with breast cancer risk [19]. Note that about 75% of the participants in that study were postmenopausal women, whereas most of our study population is premenopausal. This evidence suggests that a small portion of the association between family history of breast cancer and the risk of breast cancer is mediated by PMD, if any.

In analyses restricted to parous women, later age at first live birth was found to be associated with a higher risk of breast cancer but there was no evidence of mediation by PMD. These results were in line with the findings in premenopausal women in two previous studies [22, 23]. Although these two studies found significant mediation by PMD for the associations between later age at first birth and all invasive breast cancer among postmenopausal women, the proportion mediated (13-16%) is small. In another study by Tice et al. (2005), later age at first live birth was also found to be a significant breast cancer risk factor among women without a first-degree family history of breast cancer [56]. Adjusting for BI-RADS mammographic density categories attenuated the relative risk by about 16%. These results suggest that the association between age at first live birth and breast cancer risk is not likely to be mediated by PMD, at least among premenopausal women. The portion of mediation would be small if there is any.

The presence of inconsistent mediation for adult BMI was also observed in several studies that compared the association between BMI and breast cancer risk before and after further adjustment for breast density [16, 22, 23, 57-61]. The overall association between BMI and breast cancer risk was not significant in all these studies except for one, which showed a significantly lower risk of breast cancer risk among premenopausal women with higher BMI [16]. Further adjustment for mammographic density consistently strengthened the overall positive associations, whereas the overall negative associations largely became positive. Our results showed that BMI at age 18 had a significant negative indirect association with breast cancer risk through PMD,

although the overall association was not significant. This finding is partially supported by Rice et al. (2016) [22], who found that PMD mediated a substantial portion of the significant association between BMI at age 18 and breast cancer risk among premenopausal women. While the overall association was significant among premenopausal women, the association in postmenopausal women was not significant. Similar results were found in another study that included both pre- and post-menopausal women, which showed that the association between BMI at age 18 and breast cancer risk was only significant when comparing overweight or obese women to those with a BMI between 20 and 22.4 [61]. This significant association was substantially attenuated after adjustment for mammographic density. These results suggest that BMI at a younger age might act predominantly through its negative association through breast density, whereas BMI at an older age may also act positively through pathways independent of breast density, resulting in an inconsistent mediation.

Our study has several limitations. In order to establish a causal interpretation of the direct and indirect effects, it is important to make the no-unmeasured-confounding assumptions. Although we have checked confounding for a large number of participant characteristics, there may be other unmeasured factors (such as physical activity) that were not ascertained in our study. Our study has a relatively small sample size (547 cases and 472 controls), which may be the reason why we did not have sufficient statistical power to detect a significant association for some risk factors such as a history of breast biopsy and number of live births.

The temporal relationship, that the exposure preceded the mediator and that the mediator preceded the outcome, is also necessary. With a case-control design, our study was not able to make sure all the risk factors occurred before mammographic measurement, although screening mammograms were taken before the diagnosis of breast cancer. Since a majority of our study participants had their mammograms at age 35 years old or older, it is reasonable to assume that the reproductive factors and lifestyle habits considered in our study were established before the measurement of breast density. However, this assumption does not apply to the presence of calcifications and history of breast biopsy, because calcifications and mammographic density were measured using the same mammogram, while a biopsy may be performed after detecting a high-density pattern in the mammogram. Therefore, we conducted a sensitivity analysis by assuming

the mediation path in a way that calcifications or breast biopsies were the potential mediators for the effect of PMD. The results provided no support for the hypothesis that calcifications or breast biopsies mediated the association of PMD with breast cancer risk (Supplementary Table 4.3). However, given that women with denser breasts are more likely to undergo breast biopsy and they likely had a high PMD at the time their calcification was detected, it is difficult to determine the temporality between these two breast cancer risk factors and PMD. Therefore, the results must be interpreted with caution. Further studies are warranted to determine the causal direction of the relationships. The assumption of temporality may have a better application to cohort studies or nested case-control studies, where risk factors are ascertained before the measurement of mammographic density, both of which may have occurred prior to the development of the disease. In addition, note that the mediator we studied is percent mammographic density. We did not use "Wolfe's classification" because PMD was found to provide more information on breast cancer risk than Wolfe's parenchymal patterns and the parenchymal patterns appeared to be redundant once PMD is taken into account [62]. However, it is possible that other aspects of mammographic patterns, such as texture, coarseness, stiffness, etc., could potentially be responsible for part of the effect of these different exposures on the risk of breast cancer. These characteristics may be important but would not be captured simply by a percent mammographic density measure.

4.5 CONCLUSIONS

In summary, using data from four population-based case-control studies of women having screening mammograms under age 50, we demonstrated that breast calcifications, being parous, and higher BMI affect the risk of breast cancer partially through their effect on PMD. On the other hand, first-degree family history of breast cancer, age at first live birth, and cigarette smoking affect breast cancer risk mainly through pathways independent of PMD. These findings may provide insights into the mechanisms involved in the development of breast cancer and highlight a potential biological pathway from breast density to the etiology of breast cancer.

TABLES

Table 4.1. Characteristics of cases vs. controls by selected risk factors

Variables	Case (n=547)	Control (n=472)
STUDY		
EMF	231 (42.2%)	213 (45.1%)
OB	37 (6.8%)	31 (6.6%)
BCIA	87 (15.9%)	77 (16.3%)
WISH	192 (35.1%)	151 (32.0%)
Age at diagnosis or reference date (y)		
<40	101 (18.5%)	73 (15.5%)
40-44	254 (46.4%)	222 (47.0%)
45-49	78 (14.3%)	72 (15.3%)
50-54	89 (16.3%)	90 (19.1%)
55-59	25 (4.6%)	15 (3.2%)
Age at mammogram (y)		
<35	75 (13.7%)	42 (8.9%)
35-39	215 (39.3%)	185 (39.2%)
40-44	166 (30.3%)	173 (36.7%)
45-49	91 (16.6%)	72 (15.3%)
Age at menopause (y)		
Pre-menopausal	453 (82.8%)	387 (82.0%)
<35	41 (7.5%)	43 (9.1%)
35+	53 (9.7%)	42 (8.9%)
Race: White	531 (97.4%)	458 (97.0%)
Marital status		
Single, never married	33 (6.2%)	19 (4.1%)
Married/living as married	428 (80.3%)	370 (79.9%)
Separated/Divorced/Widowed	72 (13.5%)	74 (16.0%)
Education level		
Attended or completed HS/GED or less	120 (22.0%)	127 (26.9%)
Technical school/2-year college	55 (10.1%)	48 (10.2%)
Attended or completed college	294 (53.8%)	243 (51.5%)
Attended or completed graduate school	77 (14.1%)	54 (11.4%)
Income		
Less than median income category	153 (28.3%)	149 (32.3%)
Median income category	146 (27.0%)	121 (26.2%)
Greater than median income category	241 (44.6%)	192 (41.6%)
Drinks per week, 6 to 2 years before		
No drinking	131 (23.9%)	131 (27.8%)
<1.0 drink per week	136 (24.9%)	104 (22.0%)
1.0-2.99 drinks per week	93 (17.0%)	74 (15.7%)
3.0-6.99 drinks per week	81 (14.8%)	62 (13.1%)

Table 4.1. Characteristics of cases vs. controls by selected risk factors (cont.)

Variables	Case (n=547)	Control (n=472)
7.0+ drinks per week	106 (19.4%)	101 (21.4%)
Ever smoked cigarettes	296 (54.1%)	222 (47.0%)
First degree family had BC	120 (21.9%)	60 (12.7%)
Parity: Nulliparous	132 (24.1%)	82 (17.4%)
Ever breastfed (among parous)	284 (68.6%)	268(68.7%)
Total duration of BCPs (months)		
Never used	50 (9.3%)	50 (10.6%)
>0 - 60 months	284 (52.9%)	239 (50.7%)
>60 months	203 (37.8%)	182 (38.6%)
Estrogen use history		
Never	486 (88.8%)	414 (87.7%)
Past	9 (1.6%)	13 (2.8%)
Current	52 (9.5%)	45 (9.5%)
Progesterone ever		
No	512 (93.6%)	431 (91.3%)
Yes	35 (6.4%)	41 (8.7%)
Wolfe classification		
N1	2 (0.4%)	11 (2.3%)
P1	62 (11.3%)	135 (28.6%)
P2	420 (76.8%)	302 (64.0%)
DY	63 (11.5%)	24 (5.1%)
Calcification class		
No calcifications	192 (35.1%)	229 (48.5%)
Lowest (non-epithelial)	6 (1.1%)	11 (2.3%)
Low suspicion	138 (25.2%)	109 (23.1%)
Intermediate	77 (14.1%)	63 (13.3%)
High	100 (18.3%)	52 (11.0%)
Highest	34 (6.2%)	8 (1.7%)
Breast biopsy/aspiration/lumpectomy ever	142 (26.0%)	101 (21.4%)
Type of mammogram film		
X-ray	485 (88.7%)	429 (90.9%)
Xeroradiograph	62 (11.3%)	43 (9.1%)
Age at menarche (y)	12.49 (1.5)	12.45 (1.4)
Age at first live birth (y) (among parous)	24.4 (5.2)	23.8 (5.1)
Number of live births (among parous)	2.2 (0.9)	2.2 (1.0)
Months breastfed (among parous)	8.9 (13.9)	8.2 (11.6)
Total duration of BCPs (months)	54.0 (50.2)	56.1 (53.7)
Body Mass Index (kg/m^2)	24.5 (5.0)	25.2 (5.8)
BMI at age 18 (kg/m^2)	20.5 (2.6)	20.6 (2.9)
Percent mammographic density (%)	61.3 (21.2)	48.6 (25.1)
Time since mammogram (y)	4.8 (3.1)	4.5 (3.2)

Table 4.2. Odds ratios (ORs) and 95% CI for risk of breast cancer, adjusted or not adjusted for percent mammographic density (PMD), the difference method

	Unadjusted for PMD			Adjusted for PMD			*Proportion Explained*§	
	OR*	**95%CI**	**p-value**	**OR_{adj}†**	**95%CI**	**p-value**	***PE (95%CI)***	**p-value**
Full dataset								
BMI (continuous, kg/m^2)	0.98	0.96, 1.00	0.090	1.04	1.01, 1.07	0.015	~	~
Normal/Underweight (BMI <25)	1.00	Reference		1.00	Reference		Reference	
Overweight/obese (BMI 25+ vs <25)	0.92	0.70, 1.20	0.530	1.57	1.15, 2.16	0.005	~	~
BMI at age 18 (continuous, kg/m^2)	0.98	0.93, 1.03	0.350	1.03	0.98, 1.09	0.196	~	~
Normal/Underweight (BMI at age 18y <25)	1.00	Reference		1.00	Reference		Reference	
Overweight/obese at age 18 (BMI 25+ vs <25)	0.85	0.49, 1.45	0.540	1.29	0.73, 2.31	0.387	~	~
Breast biopsy/aspiration/lumpectomy never	1.00	Reference		1.00	Reference		Reference	
Breast biopsy/aspiration/lumpectomy ever	1.26	0.92, 1.72	0.152	1.06	0.77, 1.47	0.720	64.4% (3.9% - 98.8%)	<.001
Calcification class (continuous score)	1.26	1.16, 1.37	<.001	1.22	1.12, 1.32	<.001	16.5% (8.7% - 29.0%)	<.001
Calcification (no)	1.00	Reference		1.00	Reference		Reference	
Calcification (yes vs no)	1.86	1.43, 2.43	<.001	1.66	1.26, 2.19	<.001	18.5% (8.5% - 35.7%)	0.001
First degree family history of BC no	1.00	Reference		1.00	Reference		Reference	
First degree family history of BC yes	2.07	1.46, 2.96	<.001	2.12	1.48, 3.08	<.001	-3.8% (~)	~
Nulliparous	1.00	Reference		1.00	Reference		Reference	
Parous vs nulliparous	0.67	0.48, 0.94	0.019	0.80	0.57, 1.13	0.207	42.5% (11.4% - 81.0%)	<.001
Smoking never	1.00	Reference		1.00	Reference		Reference	
Smoking ever	1.26	0.97, 1.63	0.082	1.25	0.96, 1.63	0.099	2.4% (0.0% - 100.0%)	0.437
Parous women only‡								
Number of live births (continuous)	1.04	0.88, 1.23	0.636	1.12	0.94, 1.34	0.215	~	~
Number of live births (1-2)	1.00	Reference		1.00	Reference		Reference	
Number of live births (3+ vs 1-2)	1.08	0.77, 1.52	0.664	1.25	0.87, 1.80	0.220	~	~
Age at first live birth (continuous)	1.04	1.01, 1.08	0.011	1.04	1.01, 1.07	0.023	11.0% (0.8% - 66.4%)	0.200

Table 4.2. Odds ratios (ORs) and 95% CI for risk of breast cancer, adjusted or not adjusted for percent mammographic density (PMD), the difference method (cont.)

	Unadjusted for PMD			Adjusted for PMD			*Proportion Explained*§	
	OR*	**95%CI**	**p-value**	**OR_{adj}†**	**95%CI**	**p-value**	***PE (95%CI)***	**p-value**
Age at first live birth (15-29)	1.00	Reference		1.00	Reference		Reference	
Age at first live birth (30+ vs <30)	1.36	0.90, 2.06	0.150	1.31	0.85, 2.03	0.216	15.0% (0.4% - 89.7%)	0.255

*Multivariate analyses adjusted for study (EMF, OB, BCIA, WISH), age at mammogram (<35 y, 35-39 y, 40-44 y, 45-49 y), BMI (<20, 20 to <24, 24 to <28, 28 to 32 kg/m2), calcification (yes or no), first degree family history of breast cancer (yes or no), parity (yes or no), breast biopsy/aspiration/lumpectomy ever (yes or no), smoking (ever or never), mammogram film type (X-ray or Xeroradiograph);

†In addition to risk factors listed in footnote *, odds ratios were further adjusted for continuous percent mammographic density;

‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (1, 2, or 3+) for parity and further adjusted for age at first live birth (continuous, years);

§Proportion Explained (PE) index, percent of the total association (on the log odds scale) between the exposure and breast cancer risk that was mediated by PMD, was calculated using the following equation: $PE= 1 - (\ln OR_{adjusted}/\ln OR_{unadjusted})$; It was not calculated (notated using symbol ~) if the absolute value of PE is outside the range of [0, 1].

Table 4.3. Total, direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (PMD), among all women or parous women*

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Without exposure × MD interaction										
Full dataset†										
BMI (continuous, kg/m^2)**	1.05	1.02, 1.08	0.003	0.94	0.92, 0.96	<.001	0.98	0.96, 1.01	0.274	>
BMI (25+ vs <25)	1.57	1.15, 2.15	0.005	0.57	0.48, 0.69	<.001	0.90	0.66, 1.23	0.514	>
BMI at age 18 (continuous, kg/m^2)**	1.04	0.99, 1.10	0.121	0.93	0.91, 0.96	<.001	0.97	0.92, 1.02	0.256	>
BMI at age 18 (25+ vs <25)	1.29	0.73, 2.29	0.386	0.60	0.47, 0.77	<.001	0.78	0.42, 1.42	0.413	>
Breast biopsy/aspiration/lumpectomy	1.06	0.77, 1.47	0.720	1.28	1.11, 1.49	0.001	1.36	0.96, 1.93	0.083	83.1%
Calcification (yes vs no)	1.66	1.26, 2.19	<.001	1.16	1.04, 1.30	0.009	1.93	1.44, 2.59	<.001	29.0%
Calcification class (continuous score)	1.22	1.12, 1.32	<.001	1.05	1.01, 1.08	0.015	1.27	1.16, 1.39	<.001	20.5%
First degree family history of BC	2.12	1.47, 3.07	<.001	0.89	0.76, 1.05	0.168	1.89	1.27, 2.82	0.002	Not mediated
Parous vs nulliparous	0.80	0.57, 1.13	0.207	0.76	0.65, 0.90	0.001	0.61	0.42, 0.89	0.010	48.6%
Smoking ever	1.25	0.96, 1.63	0.099	1.02	0.92, 1.14	0.676	1.28	0.96, 1.71	0.092	Not mediated
Parous women only‡										
Number of live births (continuous)	1.12	0.94, 1.34	0.215	0.90	0.83, 0.97	0.007	1.01	0.83, 1.22	0.949	<
Age at first live birth (continuous)	1.04	1.01, 1.07	0.023	1.00	0.98, 1.01	0.876	1.04	1.00, 1.07	0.043	Not mediated
With exposure × MD interaction										
Full dataset†										
Overweight/obese (BMI 25+)	1.54	1.04, 2.29	0.033	0.58	0.46, 0.73	<.001	0.90	0.65, 1.23	0.499	>
Overweight/obese at age 18 (BMI 25+)	1.70	0.70, 4.15	0.245	0.49	0.29, 0.85	0.011	0.84	0.42, 1.67	0.612	>
Breast biopsy/aspiration/lumpectomy	1.08	0.77, 1.49	0.665	1.20	1.02, 1.39	0.023	1.29	0.91, 1.81	0.152	73.6%
Calcification (yes vs no)	1.63	1.23, 2.16	0.001	1.15	1.03, 1.28	0.012	1.87	1.37, 2.54	<.001	28.0%
Calcification class (continuous score)	1.21	1.11, 1.32	<.001	1.05	1.01, 1.09	0.016	1.27	1.16, 1.39	<.001	21.3%
First degree family history of BC	2.46	1.56, 3.88	<.001	0.86	0.68, 1.08	0.181	2.10	1.31, 3.37	0.002	Not mediated
Parous vs nulliparous	0.87	0.60, 1.25	0.439	0.75	0.64, 0.89	0.001	0.65	0.45, 0.94	0.024	61.2%
Smoking ever	1.25	0.95, 1.65	0.114	1.02	0.92, 1.14	0.677	1.28	0.95, 1.72	0.106	Not mediated

Table 4.3. Total, direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (PMD), among all women or parous women* (cont.)

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Parous women only‡										
Number of live births (continuous)	1.17	0.95, 1.44	0.144	0.91	0.84, 0.98	0.014	1.06	0.85, 1.33	0.606	<
Age at first live birth (continuous)	1.04	1.01, 1.07	0.023	1.00	0.99, 1.01	0.876	1.04	1.00, 1.08	0.038	Not mediated

*Mediation analysis using the SAS macro by Valeri, L., & VanderWeele, T. J. (2013) and the 95% CIs were estimated using the delta method;
†Multivariate analyses adjusted for study (EMF, OB, BCIA, WISH), age at mammogram (<35 y, 35-39 y, 40-44 y, 45-49 y), percent mammographic density (continuous, %), BMI (<20, 20 to <24, 24 to <28, 28 to 32 kg/m^2), calcification (yes or no), family history of breast cancer in a first-degree relative (yes or no), parity (yes or no), breast biopsy/aspiration/lumpectomy (ever or never), smoking (ever or never), mammogram film type (X-ray or Xeroradiograph);
‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (1, 2, or 3+) for parity and further adjusted for age at first live birth (continuous, years);
§Proportion mediated (PM) was calculated using the following formula if NIE is significant: PM = OR^{NDE} (OR^{NIE} - 1)/(OR^{NDE} × OR^{NIE} - 1); PM calculated to be < 0% and > 100% were denoted by "<" and ">" respectively;
**Mediation analysis with exposure-mediator interaction for continuous BMI and BMI at age 18 was not reported due to high multicollinearity detected for the interaction term;
Abbreviations: CI, confidence interval; OR, odds ratio; NDE, natural direct effect; NIE, natural indirect effect; PM, proportion mediated.

SUPPLEMENTAL MATERIAL

Supplementary Table 4.1. Total, direct and indirect effects and their 95% CI (by bootstrapping) of exposure on risk of breast cancer, mediated by percent mammographic density (MD), among all women or parous women*

	Natural Direct Effect (NDE)		Natural Indirect Effect (NIE)		Total Effect (TE)		Proportion Mediated§
	OR	95%CI	OR	95%CI	OR	95%CI	
Without exposure × MD interaction							
Full dataset†							
BMI (continuous, kg/m^2)**	1.04	1.01, 1.08	0.94	0.93, 0.96	0.98	0.96, 1.01	>
Overweight/obese (BMI 25+)	1.60	1.14, 2.20	0.57	0.46, 0.67	0.91	0.67, 1.19	>
BMI at age 18 (continuous, kg/m^2)**	1.04	0.99, 1.10	0.93	0.91, 0.95	0.97	0.92, 1.02	>
Overweight/obese at age 18 (BMI 25+)	1.38	0.73, 2.49	0.60	0.45, 0.78	0.82	0.43, 1.42	>
Breast biopsy/aspiration/lumpectomy	1.08	0.76, 1.50	1.29	1.12, 1.51	1.39	0.99, 1.91	80.1%
Calcification (yes vs no)	1.69	1.24, 2.22	1.17	1.05, 1.31	1.98	1.45, 2.62	29.7%
Calcification class (continuous score)	1.22	1.12, 1.33	1.05	1.01, 1.09	1.28	1.17, 1.40	20.3%
First degree family history of BC	2.18	1.51, 3.11	0.89	0.75, 1.03	1.94	1.32, 2.76	Not mediated
Parous vs nulliparous	0.81	0.55, 1.13	0.76	0.66, 0.88	0.62	0.41, 0.86	49.6%
Smoking ever	1.27	0.97, 1.64	1.02	0.91, 1.15	1.30	0.98, 1.68	Not mediated
Parous women only‡							
Number of live births (3+ vs 1-2)	1.29	0.88, 1.89	0.80	0.68, 0.93	1.03	0.70, 1.50	<
Number of live births (continuous)	1.13	0.93, 1.38	0.90	0.82, 0.96	1.01	0.83, 1.21	<
Age at first live birth (30+ vs <30)	1.36	0.86, 2.09	1.01	0.84, 1.20	1.37	0.86, 2.11	Not mediated
Age at first live birth (continuous)	1.04	1.00, 1.08	1.00	0.99, 1.01	1.04	1.00, 1.08	Not mediated
With exposure × MD interaction							
Full dataset†							
Overweight/obese (BMI 25+)	1.61	1.02, 2.49	0.58	0.42, 0.74	0.91	0.66, 1.22	>
Overweight/obese at age 18 (BMI 25+)	2.29	0.82, 6.27	0.48	0.17, 0.83	0.90	0.48, 1.58	<
Breast biopsy/aspiration/lumpectomy	1.10	0.80, 1.53	1.19	1.05, 1.36	1.31	0.94, 1.78	66.8%
Calcification (yes vs no)	1.65	1.22, 2.21	1.15	1.05, 1.27	1.89	1.42, 2.49	27.6%
Calcification class (continuous score)	1.22	1.12, 1.34	1.05	1.01, 1.09	1.28	1.17, 1.40	21.8%
First degree family history of BC	2.66	1.67, 4.17	0.84	0.60, 1.03	2.20	1.49, 3.20	Not mediated

Supplementary Table 4.1. Total, direct and indirect effects and their 95% CI (by bootstrapping) of exposure on risk of breast cancer, mediated by percent mammographic density (MD), among all women or parous women* (cont.)

	Natural Direct Effect (NDE)		Natural Indirect Effect (NIE)		Total Effect (TE)		Proportion Mediated§
	OR	95%CI	OR	95%CI	OR	95%CI	
Parous vs nulliparous	0.87	0.60, 1.26	0.75	0.61, 0.88	0.65	0.46, 0.89	63.0%
Smoking ever	1.27	0.94, 1.67	1.02	0.90, 1.13	1.29	0.97, 1.67	Not mediated
Parous women only‡							
Number of live births (continuous)	1.18	0.92, 1.54	0.91	0.84, 0.97	1.07	0.85, 1.35	<
Number of live births (3+ vs 1-2)	1.44	0.92, 2.20	0.77	0.58, 0.93	1.09	0.75, 1.53	<
Age at first live birth (continuous)	1.04	1.00, 1.08	1.00	0.99, 1.02	1.04	1.00, 1.08	Not mediated
Age at first live birth (30+ vs <30)	1.39	0.87, 2.14	0.99	0.81, 1.15	1.37	0.85, 2.10	Not mediated

*Mediation analysis using the SAS macro by Valeri, L., & VanderWeele, T. J. (2013) and the 95% CIs were estimated using 1000 bootstrapping;
†Multivariate analyses adjusted for study (EMF, OB, BCIA, WISH), age at mammogram (<35 y, 35-39 y, 40-44 y, 45-49 y), percent mammographic density (continuous, %), BMI (<20, 20 to <24, 24 to <28, 28 to 32 kg/m2), calcification (yes or no), family history of breast cancer in a first-degree relative (yes or no), parity (yes or no), breast biopsy/aspiration/lumpectomy (ever or never), smoking (ever or never), mammogram film type (X-ray or Xeroradiograph);
‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (1, 2, or 3+) for parity and further adjusted for age at first live birth (continuous, years);
§Proportion mediated (PM) was calculated using the following formula if NIE is significant: PM = OR^{NDE} (OR^{NIE} - 1)/(OR^{NDE} × OR^{NIE} - 1); PM calculated to be < 0% and > 100% were denoted by "<" and ">" respectively;
**Mediation analysis with exposure-mediator interaction for continuous BMI and BMI at age 18 was not reported due to high multicollinearity detected for the interaction term;
Abbreviations: CI, confidence interval; OR, odds ratio; NDE, natural direct effect; NIE, natural indirect effect; PM, proportion mediated.

Supplementary Table 4.2. Direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (MD), excluding postmenopausal women at mammogram date*

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Without exposure × MD interaction										
Full dataset†										
BMI (continuous, kg/m^2)**	1.04	1.00, 1.08	0.016	0.94	0.92, 0.96	0.000	0.98	0.95, 1.01	0.279	>
Overweight/obese (BMI 25+)	1.62	1.15, 2.30	0.006	0.58	0.48, 0.71	0.000	0.95	0.67, 1.33	0.762	>
BMI at age 18 (continuous, kg/m^2)**	1.04	0.98, 1.10	0.192	0.93	0.90, 0.95	0.000	0.96	0.91, 1.02	0.192	>
Overweight/obese at age 18 (BMI 25+)	1.21	0.66, 2.24	0.536	0.56	0.43, 0.74	0.000	0.68	0.36, 1.29	0.240	>
Breast biopsy/aspiration/lumpectomy	1.21	0.85, 1.74	0.294	1.22	1.04, 1.43	0.016	1.48	1.00, 2.18	0.050	55.3%
Calcification (yes vs no)	1.85	1.36, 2.51	0.000	1.22	1.07, 1.38	0.003	2.24	1.61, 3.11	0.000	31.9%
Calcification class (continuous score)	1.24	1.13, 1.36	0.000	1.05	1.01, 1.10	0.015	1.30	1.18, 1.44	0.000	21.2%
First degree family history of BC	2.24	1.50, 3.36	0.000	0.88	0.73, 1.05	0.151	1.97	1.27, 3.05	0.003	Not mediated
Parous vs nulliparous	0.79	0.54, 1.16	0.225	0.78	0.66, 0.93	0.005	0.62	0.41, 0.93	0.022	45.1%
Smoking ever	1.30	0.97, 1.75	0.082	1.00	0.89, 1.13	0.992	1.30	0.94, 1.79	0.107	Not mediated
Parous women only‡										
Number of live births (continuous)	1.05	0.86, 1.29	0.626	0.89	0.81, 0.97	0.010	0.94	0.75, 1.16	0.552	>
Age at first live birth (continuous)	1.03	1.00, 1.07	0.083	1.00	0.98, 1.01	0.841	1.03	0.99, 1.07	0.132	Not mediated
With exposure × MD interaction										
Full dataset†										
Overweight/obese (BMI 25+)	1.64	1.05, 2.57	0.031	0.58	0.45, 0.75	0.000	0.95	0.66, 1.37	0.790	>
Overweight/obese at age 18 (BMI 25+)	1.82	0.66, 5.03	0.251	0.41	0.21, 0.79	0.008	0.74	0.35, 1.58	0.437	>
Breast biopsy/aspiration/lumpectomy	1.21	0.84, 1.74	0.297	1.178	1.00, 1.39	0.053	1.43	0.97, 2.11	0.073	50.5%
Calcification (yes vs no)	1.78	1.29, 2.45	0.000	1.176	1.04, 1.32	0.008	2.09	1.48, 2.95	0.000	28.6%
Calcification class (continuous score)	1.23	1.11, 1.35	0.000	1.055	1.01, 1.10	0.015	1.30	1.17, 1.44	0.000	23.1%
First degree family history of BC	2.78	1.63, 4.73	0.000	0.821	0.62, 1.08	0.165	2.28	1.32, 3.93	0.003	Not mediated
Parous vs nulliparous	0.864	0.58, 1.29	0.477	0.770	0.64, 0.93	0.005	0.665	0.44, 1.00	0.049	59.3%
Smoking ever	1.280	0.94, 1.74	0.117	0.999	0.89, 1.12	0.992	1.279	0.92, 1.78	0.145	Not mediated

Supplementary Table 4.2. Direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (MD), excluding postmenopausal women at mammogram date* (cont.)

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Parous women only‡										
Number of live births (continuous)	1.17	0.93, 1.47	0.176	0.92	0.85, 1.00	0.039	1.08	0.86, 1.36	0.515	<
Age at first live birth (continuous)	1.03	0.99, 1.07	0.167	1.00	0.98, 1.02	0.842	1.03	0.98, 1.08	0.264	Not mediated

*Mediation analysis using the method and SAS macro by Valeri, L., & VanderWeele, T. J. (2013) and the 95% CIs were estimated using the delta method;
†Multivariate analyses adjusted for study (EMF, OB, BCIA, WISH), age at mammogram (<35 y, 35-39 y, 40-44 y, 45-49 y), percent mammographic density (continuous, %), BMI (<20, 20 to <24, 24 to <28, 28 to 32 kg/m^2), calcification (yes or no), family history of breast cancer in a first-degree relative (yes or no), parity (yes or no), breast biopsy/aspiration/lumpectomy (ever or never), smoking (ever or never), mammogram film type (X-ray or Xeroradiograph);
‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (1, 2, or 3+) for parity and further adjusted for age at first live birth (continuous, years);
§Proportion mediated (PM) was calculated using the following formula if NIE is significant: PM = OR^{NDE} (OR^{NIE} - 1)/(OR^{NDE} × OR^{NIE} - 1); PM calculated to be < 0% and > 100% were denoted by "<" and ">" respectively;
**Mediation analysis with exposure-mediator interaction for continuous BMI and BMI at age 18 was not reported due to high multicollinearity detected for the interaction term;
Abbreviations: CI, confidence interval; OR, odds ratio; NDE, natural direct effect; NIE, natural indirect effect; PM, proportion mediated.

Supplementary Table 4.3. Total, direct and indirect effects of percent mammographic density (PMD) on the risk of breast cancer, mediated by breast biopsy or calcification, among all women*

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Without exposure × MD interaction										
Full dataset†										
Breast biopsy/aspiration/lumpectomy	1.03	1.02, 1.03	0.000	1.00	1.00, 1.00	0.727	1.03	1.02, 1.03	0.000	Not mediated
Calcification (yes vs no)	1.03	1.02, 1.03	0.000	1.00	1.00, 1.00	0.021	1.03	1.02, 1.04	0.000	5.6%
With exposure × MD interaction										
Full dataset†										
Breast biopsy/aspiration/lumpectomy	1.03	1.02,1.04	0.000	1.00	1.00,1.00	0.278	1.03	1.02, 1.04	0.000	Not mediated
Calcification (yes vs no)	1.03	1.02, 1.03	0.000	1.00	1.00, 1.00	0.081	1.03	1.02, 1.04	0.000	Not mediated

*Mediation analysis using the method and SAS macro by Valeri, L., & VanderWeele, T. J. (2013) and the 95% CIs were estimated using the delta method;
†Multivariate analyses adjusted for study (EMF, OB, BCIA, WISH), age at mammogram (<35 y, 35-39 y, 40-44 y, 45-49 y), percent mammographic density (continuous, %), BMI (<20, 20 to <24, 24 to <28, 28 to 32 kg/m2), calcification (yes or no), family history of breast cancer in a first-degree relative (yes or no), parity (yes or no), breast biopsy/aspiration/lumpectomy (ever or never), smoking (ever or never), mammogram film type (X-ray or Xeroradiograph);
‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (1, 2, or 3+) for parity and further adjusted for age at first live birth (continuous, years);
§Proportion mediated (PM) was calculated using the following formula if NIE is significant: $PM = OR^{NDE}(OR^{NIE} - 1)/(OR^{NDE} \times OR^{NIE} - 1)$;
**Mediation analysis with exposure-mediator interaction for continuous BMI and BMI at age 18 was not reported due to high multicollinearity detected for the interaction term;
Abbreviations: CI, confidence interval; OR, odds ratio; NDE, natural direct effect; NIE, natural indirect effect; PM, proportion mediated.

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CHAPTER 5: STUDY 2

A Mediation Analysis of the Mayo Mammography Health Study Data

Abstract

Background: Percent mammographic density (PMD) is a strong risk factor for breast cancer. Less is known about the role of PMD as an intermediate marker for breast cancer risk.

Methods: Data from a nested case-control study of breast cancer, including 677 cases and 1284 matched controls, was analyzed using mediation analysis. We estimated the direct effects of various risk factors on risk of breast cancer and their indirect effects (i.e. effects mediated through PMD), as well as the proportion mediated by PMD.

Results: The association between prior breast biopsy and risk of breast cancer was partially mediated by PMD (proportion mediated = 19.12%) in postmenopausal women, with an indirect-effect odds ratio of 1.09 (95% CI: 1.02-1.17; P = 0.016). PMD mediated 32.13% and 14.97% of the increased risks associated with combined current use of estrogen and progesterone and the number of alcoholic drinks per month, which yielded an indirect-effect odds ratio of 1.17 (95% CI: 1.02-1.33; P = 0.021) and (OR_{NIE}, 1.00; 95% CI, 1.00-1.01; P = 0.096), respectively. No significant mediation by PMD was observed with respect to a first-degree family history of breast cancer and age at menopause. There was inconsistent mediation for the effect of adult body mass index.

Conclusions: PMD partially mediated the associations between prior breast biopsy and hormone replacement therapy of combined estrogen and progestin with the risk of breast cancer among postmenopausal women, suggesting that these risk factors at least partially influence breast cancer risk through changes in breast tissue composition.

5.1 INTRODUCTION

Several independent observations suggest that mammographic breast density may be a potential mediator for breast cancer risk. First, high breast density on a mammogram has been repeatedly shown to be one of the strongest independent risk factors for breast cancer [1-4]. Second, mammographic density has also been shown to be associated with a wide array of risk factors for breast cancer such as age, menopausal status, family history of breast cancer, parity, age at first live birth, body mass index, physical activity, alcohol consumption, and hormone replacement therapy [1]. Furthermore, breast density can even be changed by several exposures or interventions that are also known to influence breast cancer risk [5]. For example, tamoxifen, an anti-estrogen, has been reported to reduce mammographic density as well as the risk of breast cancer [6-9]. In this context, it was proposed that some established breast cancer risk factors may be mediated by their intermediate effects on the mammary tissue, which is evaluated by mammographic densities.

To test this hypothesis, several studies have attempted to assess the potential role of mammographic density as a mediator for breast cancer risk [10-16]. Results showed that mammographic density partially mediated the associations for some breast cancer risk factors such as childhood somatotype, being parous, history of benign breast disease, and hormone replacement therapy (HRT) use, but not risk factors such as a family history of breast cancer. While these studies have examined whether mammographic density mediates the associations with breast cancer risk for some risk factors, the extent of mediation was not estimated using statistical mediation analyses based on a counterfactual framework. Most of the studies regarding the role of mammographic density as a mediator were based on analysis using the traditional "difference method" [10, 13-15]. To our knowledge, only two studies [13, 15] used causal mediation analysis to evaluate the effect of a number of known risk factors for breast cancer through mammographic density and one of which was conducted as a secondary analysis [13].

In the present study, we conducted a causal mediation analysis [17] using data from the Mayo Mammographic Health Study, aiming to evaluate and quantify the extent to which mammographic density mediated the association between various risk factors of interest, each factor at a time, with the risk of breast cancer risk.

5.2 METHODS

Study Design and Participants

We included data from a nested case-control study of breast cancer, the Mayo Mammography Health Study (MMHS). The data source, study design, and participant characteristics have been described in detail previously [18]. The MMHS prospectively enrolled patients scheduled for a screening mammogram from October 2003 through September 2006 at the Mayo Clinic in Rochester, MN. Women were invited to participate if they were at least 35 years old, residents of Minnesota, Iowa, or Wisconsin (tri-state), and had no personal history of breast cancer. Women scheduled for a diagnostic mammogram (known or suspected breast cancer) were not eligible. Eligible women were mailed an invitation packet consisting of a study brochure, a consent form, a baseline questionnaire, and a permission form to link to state tumor registries. This study included 677 cases of women with an initial diagnosis of either in situ or invasive disease and 1284 matched controls. Controls were randomly sampled from the underlying cohort and were matched to cases on age, year of examination, and state of residence. This study was approved by the Institutional Review Boards at Mayo Clinic (Rochester, MN).

Measurement of Mammographic Density

For all cases and women in the sub-cohort, we obtained and digitized one view from the enrollment screen-film mammogram (2003-2006). Screen-film mammograms were digitized on the Array 2905 laser digitizer. Absolute dense area, absolute non-dense area (the total area minus the dense area), and percent mammographic density (PMD, the dense area divided by the total area, times 100%) were measured from digitized images of the craniocaudal mammogram view using the Cumulus software for computer-assisted thresholding (Canto Software, San Francisco, CA, USA). PMD was estimated from the contralateral breast for cases and the corresponding side for matched controls. All images had identifying information removed and re-oriented so that all images were presented consistently despite the side evaluated. Thus, the reader was blinded to cancer status.

In addition, the clinical BI-RADS four-category tissue composition assessment, corresponding to the enrollment mammogram, was obtained from the Mayo Clinic electronic medical record. Mayo Clinic attending radiologists classified each mammogram into one of four categories as defined in the BI-RADS lexicon during this period (American College of Radiology, 3rd edition): a) the breast is almost entirely fat; b) there are scattered fibro glandular densities; c) the breast tissue is heterogeneously dense, which may lower the sensitivity of mammography; and d) the breast is extremely dense, which could obscure a lesion on mammography. All four mammogram views (craniocaudal and mediolateral oblique for ipsilateral and contralateral sides) contributed to the assessment of BI-RADS composition.

Breast cancer risk factors and confounders

All women were asked to complete a written questionnaire that covered mammogram screening behaviors; menstrual and reproductive factors; surgeries of the breast, ovaries, and/or uterus; use of hormone replacement therapies; medical history; family size and cancer history; use of non-steroidal anti-inflammatory medications; use of vitamins and complementary medicines; alcohol and cigarette use; physical activity; current weight and weight history; race; and education. Height and weight were also abstracted from the Mayo Clinic medical record at the medical visit closest in time to when each mammogram was collected for the study. Information on the selected risk factors and covariates were obtained from both medical record review and self-administered questionnaires at the time of mammography.

Statistical Methods

The mean and standard deviation (SD) were presented for continuous variables while numbers and percentages were presented for categorical variables. A causal mediation analysis was conducted following the approach outlined by VanderWeele and Vansteelandt [19], implemented by using a SAS macro that can accommodate the case-control design [20]. First, an unconditional logistic regression model for breast cancer was fitted on the potential risk factor and PMD, adjusting for matching variables and the baseline covariates. Second, a linear regression was fit for PMD on the potential risk factor and covariates using the controls. The regression for PMD and the regression for breast cancer risk were combined to obtain the ORs and 95% CIs for the following effects: (a) the natural direct effect (NDE) (i.e., the effect of the exposure on breast

cancer risk not through PMD if PMD was fixed at the level that it would have been without the exposure), (b) the natural indirect effect (NIE) (i.e., the effect of the exposure on breast cancer risk through PMD), and (c) the total association between the exposure and breast cancer risk. Despite a lack of statistical significance on all exposure–PMD interaction terms, we compared the results with and without an exposure × PMD interaction term. We used both the delta method and bootstrap sampling (1000 samples) to obtain 95% confidence intervals (CIs). The proportion mediated was estimated by the equation of $OR^{NDE} \times (OR^{NIE} -1)/(OR^{NDE} \times OR^{NIE} -1)$, where OR^{NDE} is the direct-effect odds ratio and OR^{NIE} is the indirect-effect odds ratio [19]. For all analyses, PMD measures were square-root transformed to improve normality. The analyses were conducted on post- and pre-menopausal women separately. All the p-values used were two-sided. Type I errors were set at 0.05. All statistical analyses were performed using SAS version 9.3 (SAS Institute, Inc., Cary, North Carolina, USA).

Sensitivity Analysis

To assess the robustness of our results, we repeated the analyses by restricting the data based on menopausal status at the enrollment date. This increased the sample size of premenopausal women and gave the analysis a greater power. Analyses were also conducted based on both models with and without an exposure × PMD interaction.

5.3 RESULTS

The characteristics of the study population and the set of potential risk factors and selected confounders are shown in Table 5.1. We identified a total of 537 cases and 1021 controls who were postmenopausal at the time of screening mammograms. Only 20.6% of the subjects (140/263 cases/controls) were premenopausal. The participants were largely white women (98%).

Among postmenopausal women, several risk factors were found to be significantly associated with increased risk of breast cancer (Table 5.2). These include those women with a first-degree family history of breast cancer (OR = 1.451; 95% CI: 1.132, 1.858), history of breast biopsy/lumpectomy (OR = 1.815; 95% CI: 1.410, 2.335), per year increase in age at menopause (OR = 1.126, 95% CI: 1.011, 1.256), combined estrogen and progesterone hormone replacement

therapy (HRT) use (OR = 1.932, 95% CI: 1.212, 3.070), an increasing number of alcoholic drinks (OR = 1.010; 95% CI: 1.000, 1.020), and higher BMI (OR = 1.032; 95% CI: 1.014, 1.051). Further adjustment for PMD attenuated the associations for a history of breast biopsy/lumpectomy, combined estrogen and progesterone HRT use, and the number of alcoholic drinks (Table 5.2). Among premenopausal women, no risk factor was significant. This is likely due to low power for the small sample size with only 140 cases and 263 controls. Therefore, a sensitivity analysis including subjects who were premenopausal at enrollment (191 cases and 356 controls) was conducted, which was able to detect a significant effect for having a first-degree family history of breast cancer (OR = 1.751; 95% CI: 1.097, 2.789) (Table 5.3). Further adjustment for PMD did not attenuate the association between first-degree family history and risk of breast cancer.

The results of mediation analyses for postmenopausal women are summarized in Table 5.4 and Supplementary Table 5.1. Women with a first-degree family history of breast cancer were found to have a significantly higher risk of breast cancer ($OR_{\text{total effect}}$, 1.40; 95% CI, 1.082-1.810; P = 0.010). However, no significant mediation by PMD was observed for this variable (OR_{NIE} 0.95, 95% CI: 0.902-1.011; P = 0.170). Having a history of breast biopsy/lumpectomy was indirectly associated with increased risk of breast cancer through its effect on PMD (OR_{NIE}, 1.09; 95% CI, 1.025-1.166; P = 0.007), with a proportion of 18.7% mediated by PMD. It was also directly associated with breast cancer risk (OR_{NDE}, 1.64; 95% CI, 1.269-2.120; P < 0.001) via a pathway independent of PMD. Women who reported usage of combined estrogen and progesterone HRT had a significantly increased risk of breast cancer ($OR_{\text{total effect}}$, 1.95; 95% CI, 1.203-3.160; P = 0.007). This association was decomposed into a significant indirect-effect odds ratio of 1.19 (95% CI, 1.048-1.340; P = 0.007) through PMD and a direct-effect odds ratio of 1.65 (95% CI, 1.026-2.637; P = 0.039) independent of PMD. Overall, 32.5% of the increased risk of breast cancer related to using combined estrogen and progesterone HRT was attributable to higher PMD. PMD partially mediated the association with number of alcoholic drinks (OR_{NIE}, 1.00; 95% CI, 1.00-1.01; P = 0.096) but not age at menopause (OR_{NIE}, 1.02; 95% CI, 0.92-1.14; P = 0.676).

BMI at enrollment before a questionnaire was provided displayed a highly significant negative indirect association with breast cancer risk through PMD (OR_{NIE}, 0.940; 95% CI, 0.92-0.96; P=0.000), while it displayed a significant positive relationship with breast cancer risk

(OR_{NDE}, 1.048; 95% CI, 1.02-1.08; P=0.003) independent of PMD. The opposing direct and indirect effects resulted in an overall non-significant total effect of BMI ($OR_{total\ effect}$, 0.98; 95% CI, 0.96-1.01; P = 0.274).

BMI at time of mammogram (enrollment) was associated with risk of breast cancer ($OR_{total\ effect}$, 1.03; 95% CI, 1.009-1.046; P = 0.004). It had a significant negative indirect association with breast cancer risk through PMD (OR_{NIE}, 0.931; 95% CI, 0.91-0.96; P = 0.000). However, the direct association acting independently of PMD was not significant, although positive (OR_{NDE}, 1.04; 95% CI, 0.99-1.10; P = 0.121).

The results of mediation analyses for premenopausal women are summarized in Table 5.5. Similarly, a first-degree family history of breast cancer was found to be a significant risk factor for breast cancer ($OR_{total\ effect}$, 1.72; 95% CI, 1.065-2.784; P = 0.027). However, the association was not mediated through PMD (OR_{NIE}, 0.98; 95% CI, 0.910-1.062; P = 0.664).

Including versus excluding the exposure-mediator interaction terms did not change the estimates of the direct and indirect effects dramatically (Table 5.4-5.5). When the analyses for postmenopausal women excluding those who became postmenopausal between enrollment and the time of mammograms, results were similar to that seen for all women at the time of enrollment (Supplementary Table 5.1).

5.4 DISCUSSION

Our results showed that PMD partially mediated the association between prior history of a breast biopsy and current combined hormone replacement therapy (E+P) with the risk of breast cancer among postmenopausal women. However, it did not mediate the observed association between first-degree family history of breast cancer and the risk of breast cancer in both pre- and post-menopausal women.

The present study estimated that about 19% of the increased risk associated with a history of breast biopsy is mediated through PMD among postmenopausal women. This observation of

significant mediation by PMD for the association between prior breast biopsy and breast cancer risk in postmenopausal is consistent with a previous study that included the MMHS as part of the data source [14]. In the study, a significant mediation by PMD of the association between prior breast biopsy and invasive breast cancer risk was found in both pre- and postmenopausal women, with mediation proportions of 17% and 24% respectively. In another study using data from the Nurses' Health Study (NHS), PMD was also found to mediate the association between history of biopsy-confirmed benign breast disease and breast cancer risk, with 17% and 33% mediated in pre- and postmenopausal women respectively [13]. The observed partial mediation is also supported by an earlier study [21] that compared the relative risks of Gail model risk factors before and after adjusting for BI-RADS mammographic density categories, which showed that the association of having a previous biopsy with breast cancer was reduced by 13% and 19% for women under age 50 and those aged 50 or above respectively. Although significant mediation by PMD of the association between prior breast biopsy and breast cancer risk was consistently observed in these studies, this result should be cautiously interpreted. This is because it is difficult to determine the temporality between breast biopsy and PMD and a biopsy may be requested after detecting a high-density pattern in the mammogram. However, in our study, breast biopsy was assessed prior to PMD measurements.

PMD was found to partially mediate the association between current use of combined estrogen and progestin HRT and the risk of breast cancer in postmenopausal women, with a mediated proportion of 32%. This result is in agreement with that of Rice et al., who analyzed data by pooling MMHS data with the other three case-control studies and found that 26% of the increased risk associated with current estrogen plus progestin HRT use was mediated by PMD. A study by Byrne et al. even found that the increase in breast cancer risk among postmenopausal women using estrogen plus progestin HRT regimen was completely mediated by the increase in PMD after a year of HRT treatment [16]. In our study, the total effects for the current use of any HRT and current estrogen-only HRT use were not significant. Thus, we did not present the mediated proportion. These results are supported by previous clinical trials that have demonstrated that postmenopausal treatment with formulations of estrogen plus progestin, is associated with an increase in mammographic density and risk of breast cancer [22], whereas estrogen therapy alone is not [23].

A family history of breast cancer in first-degree relatives is an established risk factor for breast cancer. In our study, the increased risk of breast cancer in relation to a first-degree family history of breast cancer was found to be independent of PMD. This result of no mediation is consistent with two recent studies which showed that the association with a family history of breast cancer was not mediated by PMD in both premenopausal and postmenopausal women [13, 14]. However, in another study, PMD was found to explain 14% (95% CI, 4-39%) of the association of family history (at least one affected first-degree relative) with breast cancer risk [10]. This evidence suggests that only a small portion of the association between family history of breast cancer and the risk of breast cancer may be mediated by PMD if any.

We hypothesize that BMI at a younger age might act predominantly through its negative association through breast density, whereas BMI at an older age may also act positively through pathways independent of breast density. Thus, for young women, BMI is a protective factor acting mainly through reducing breast density. However, for older women (postmenopausal), other pathways may play a more important role, thus BMI is a risk factor for postmenopausal women. Although the BMI measures were not significantly associated with risk of breast cancer in our data, results from casual mediation analyses suggested the presence of inconsistent mediation or suppression, in which the direct and indirect effects had opposite directions while the total effect was not significant.

Limitations of the study recognized by the authors include the small number of premenopausal women, the uncertain temporality for breast biopsy and mammograms, and the representativeness of the controls which are a random sample of the population in a nested case-control design. In addition, the mediation results are based on PMD but not on other mammographic measures available in the study, including absolute dense area and absolute non-dense area by the Cumulus software and the clinical BI-RADS four-category tissue composition assessment. We used PMD as the potential mediator because it was found to be a stronger breast cancer risk factor than the absolute dense area and non-dense area [3, 24]. Another reason is that previous studies found that Tamoxifen can reduce PMD and breast cancer risk, however, it is unknown whether a decrease in the dense area, an increase in the nondense area, or both were

responsible for the change in PMD (Norman F Boyd 2011). Therefore, it makes sense to examine if PMD serves as a mediator for breast cancer risk before conducting further mediation analysis on other potential mediators.

5.5 CONCLUSIONS

In summary, the present study demonstrated that prior breast biopsy and current combined hormone replacement therapy (E+P) may affect the risk of breast cancer partially through their effect on PMD among postmenopausal women. On the other hand, a first-degree family history of breast cancer and the number of alcoholic drinks per month affect breast cancer risk mainly through pathways independent of PMD. These findings may provide insights into the mechanisms involved in the development of breast cancer and highlight a potential biological pathway through breast density to the etiology of breast cancer.

TABLES

Table 5.1. Selected risk factors at the time of mammography by case/control status and menopausal status

	Postmenopausal		Premenopausal	
	Cases (N=537)	**Controls (N=1021)**	**Cases (N=140)**	**Controls (N=263)**
Mean (SD)				
Age (years)	63.8 (9.1)	63.5 (9.5)	46.8 (5.6)	46.5 (5.1)
Percent mammography density (%)	16.2 (11.2)	13.8 (10.9)	25.6 (12.8)	22.4 (15.5)
Dense area (cm^2)	23.7 (16.9)	19.5 (16.5)	31.5 (16.3)	27.2 (17.1)
Non-dense area (cm^2)	141.7 (65.9)	141.5 (67.9)	106.3 (62.5)	119.5 (71.9)
Weight (LBS)	167.6 (36.1)	161.6 (36.4)	161.0 (36.8)	166.7 (43.3)
Height (Inches)	64.8 (2.4)	64.5 (2.4)	65.4 (2.8)	65.2 (2.6)
BMI (kg/m^2)	29.2 (6.4)	28.1 (6.1)	27.0 (6.1)	28.0 (7.2)
Number of alcoholic drinks (# per month)	6.6 (12.3)	5.7 (10.3)	5.8 (9.1)	5.9 (9.2)
Godin Scale Score	22.0 (17.7)	22.7 (18.7)	28.7 (22.9)	27.7 (21.0)
***N* (percent)**				
Race or ethnicity				
White	494 (99.2%)	948 (98.6%)	127 (98.4%)	245 (98.0%)
Other	4 (0.8%)	13 (1.4%)	2 (1.6%)	5 (2.0%)
Education				
Grade school or junior high	5 (1.0%)	10 (1.0%)	1 (0.8%)	2 (0.8%)
High school	132 (26.9%)	291 (30.5%)	11 (8.5%)	33 (13.2%)
College	253 (51.5%)	478 (50.1%)	96 (74.4%)	169 (67.6%)
Professional (after college)	101 (20.6%)	175 (18.3%)	21 (16.3%)	46 (18.4%)
State				
Minnesota	421 (78.4%)	799 (78.3%)	122 (87.1%)	236 (89.7%)
Wisconsin	34 (6.3%)	50 (4.9%)	4 (2.9%)	6 (2.3%)
Iowa	42 (7.8%)	107 (10.5%)	4 (2.9%)	8 (3.0%)
Unspecified	40 (7.4%)	65 (6.4%)	10 (7.1%)	13 (4.9%)
BIRADS				
1	96 (17.9%)	273 (26.7%)	13 (9.3%)	42 (16.0%)
2	243 (45.3%)	427 (41.8%)	34 (24.3%)	89 (33.8%)
3	171 (31.8%)	280 (27.4%)	67 (47.9%)	101 (38.4%)
4	27 (5.0%)	41 (4.0%)	26 (18.6%)	31 (11.8%)
Previous breast biopsy				
No breast surgery	372 (69.5%)	816 (80.1%)	122 (89.7%)	238 (91.5%)
Breast biopsy or lumpectomy	159 (29.7%)	191 (18.7%)	14 (10.3%)	19 (7.3%)
Other breast surgery including mastectomy	4 (0.7%)	12 (1.2%)	0 (0.0%)	3 (1.2%)
Family history of breast cancer				
No	383 (71.3%)	798 (78.2%)	111 (79.3%)	227 (86.3%)
Yes	154 (28.7%)	223 (21.8%)	29 (20.7%)	36 (13.7%)
Age at menarche				
9 or younger	7 (1.3%)	6 (0.6%)	2 (1.5%)	1 (0.4%)
10	22 (4.1%)	33 (3.2%)	4 (2.9%)	5 (1.9%)
11	66 (12.3%)	117 (11.5%)	11 (8.1%)	37 (14.2%)

Table 5.1. Selected risk factors at the time of mammography by case/control status and menopausal status (cont.)

	Postmenopausal		Premenopausal	
	Cases (N=537)	**Controls (N=1021)**	**Cases (N=140)**	**Controls (N=263)**
12	139 (26.0%)	265 (26.0%)	29 (21.3%)	76 (29.2%)
13	156 (29.2%)	295 (28.9%)	52 (38.2%)	70 (26.9%)
14	64 (12.0%)	136 (13.3%)	16 (11.8%)	30 (11.5%)
15 or older	46 (8.6%)	103 (10.1%)	12 (8.8%)	28 (10.8%)
Unknown	35 (6.5%)	64 (6.3%)	10 (7.4%)	13 (5.0%)
Nulliparous				
No	466 (88.6%)	879 (87.8%)	120 (87.0%)	227 (87.0%)
Yes	60 (11.4%)	122 (12.2%)	18 (13.0%)	34 (13.0%)
Parity (among parous)				
1	45 (9.7%)	88 (10.0%)	14 (11.7%)	31 (13.7%)
2	159 (34.1%)	295 (33.6%)	56 (46.7%)	119 (52.4%)
3	131 (28.1%)	256 (29.1%)	35 (29.2%)	49 (21.6%)
4	67 (14.4%)	130 (14.8%)	13 (10.8%)	23 (10.1%)
5	64 (13.7%)	110 (12.5%)	2 (1.7%)	5 (2.2%)
Age at first birth (among parous)				
<30	392 (89.7%)	763 (91.6%)	82 (73.2%)	166 (77.2%)
30+	45 (10.3%)	70 (8.4%)	30 (26.8%)	49 (22.8%)
Number of children breastfed for at least a month (among parous)				
Did not breastfeed any	221 (50.7%)	417 (50.0%)	33 (29.7%)	70 (32.7%)
1 to 2 children	137 (31.4%)	261 (31.3%)	46 (41.4%)	106 (49.5%)
3 to 5 children	71 (16.3%)	140 (16.8%)	31 (27.9%)	36 (16.8%)
6 to 11 children or more	7 (1.6%)	14 (1.7%)	0 (0.0%)	1 (0.5%)
Unknown	0 (0.0%)	2 (0.2%)	1 (0.9%)	1 (0.5%)
Postmenopausal at enrollment				
No	56 (10.4%)	96 (9.4%)	140 (100.0%)	263 (100.0%)
Yes	481 (89.6%)	925 (90.6%)	0 (0.0%)	0 (0.0%)
Age at menopause				
<30	3 (0.7%)	16 (2.0%)	-	-
30-39	33 (8.2%)	92 (11.7%)	-	-
40-44	49 (12.2%)	101 (12.8%)	-	-
45-49	106 (26.3%)	194 (24.7%)	-	-
50-54	163 (40.4%)	289 (36.7%)	-	-
55+	49 (12.2%)	95 (12.1%)	-	-
Birth control pill use				
Never	189 (35.3%)	346 (34.0%)	18 (13.2%)	23 (8.8%)
Yes, Past	333 (62.2%)	651 (63.9%)	96 (70.6%)	190 (73.1%)
Yes, Current	12 (2.2%)	15 (1.5%)	21 (15.4%)	47 (18.1%)
Yes, Unknown	1 (0.2%)	7 (0.7%)	1 (0.7%)	0 (0.0%)
Hormone replacement therapy use				
Never	162 (30.3%)	310 (30.4%)	126 (92.6%)	231 (88.8%)
Past	238 (44.5%)	450 (44.2%)	5 (3.7%)	15 (5.8%)

Table 5.1. Selected risk factors at the time of mammography by case/control status and menopausal status (cont.)

	Postmenopausal		Premenopausal	
	Cases (N=537)	**Controls (N=1021)**	**Cases (N=140)**	**Controls (N=263)**
Current, E only	79 (14.8%)	168 (16.5%)	0 (0.0%)	2 (0.8%)
Current, P only	0 (0.0%)	1 (0.1%)	2 (1.5%)	1 (0.4%)
Current, E+P	40 (7.5%)	44 (4.3%)	1 (0.7%)	9 (3.5%)
Current, other	4 (0.7%)	21 (2.1%)	1 (0.7%)	0 (0.0%)
Current, unknown	12 (2.2%)	25 (2.5%)	1 (0.7%)	2 (0.8%)
Regular alcohol use				
No	167 (31.2%)	348 (34.2%)	30 (22.1%)	61 (23.5%)
Yes	368 (68.8%)	671 (65.8%)	106 (77.9%)	199 (76.5%)
Smoking history				
Never	327 (61.1%)	626 (61.4%)	84 (61.8%)	173 (66.5%)
Yes, Past	188 (35.1%)	334 (32.8%)	42 (30.9%)	68 (26.2%)
Yes, Current	20 (3.7%)	59 (5.8%)	10 (7.4%)	19 (7.3%)
Frequency to work up a sweat				
Often	73 (14.9%)	164 (17.2%)	28 (21.5%)	55 (22.3%)
Sometimes	271 (55.2%)	503 (52.9%)	64 (49.2%)	124 (50.2%)
Never/Rarely	147 (29.9%)	284 (29.9%)	38 (29.2%)	68 (27.5%)
Godin Scale				
Score <14 Sedentary	168 (33.9%)	334 (34.9%)	36 (27.7%)	69 (27.8%)
Score 14-23 Moderate	131 (26.5%)	221 (23.1%)	28 (21.5%)	51 (20.6%)
Score ≥24 Active	196 (39.6%)	402 (42.0%)	66 (50.8%)	128 (51.6%)

BMI: body mass index, E: estrogen, E + P: estrogen plus progestin

[a] Among parous

Table 5.2. Odds ratios (ORs) for breast cancer risk unadjusted and adjusted for PMD in women who were postmenopausal at the time of mammogram

Selected risk factor	Cases / controls	OR (95% CI) Unadjusted for PMD	OR (95% CI) Adjusted for PMD
BMI (kg/m^2)	524/999	**1.032 (1.014, 1.051)**	**1.063 (1.042, 1.085)**
Height (inch) (adjust for BMI)	524/999	1.039 (0.992, 1.088)	1.043 (0.995, 1.093)
Height (inch) (adjust for weight)	525/997	1.019 (0.971, 1.069)	1.000 (0.953, 1.051)
Weight (lbs) (adjust for height)	525/997	**1.005 (1.002, 1.008)**	**1.010 (1.007, 1.014)**
BMI (kg/m^2), current	535/1017	**1.029 (1.010, 1.049)**	**1.062 (1.040, 1.085)**
BMI (kg/m^2), 5 years ago	530/1008	1.012 (0.993, 1.031)	**1.036 (1.015, 1.058)**
BMI (kg/m^2), 10 years ago	525/1004	1.019 (0.998, 1.040)	**1.046 (1.022, 1.070)**
Age at menarche ≤10 vs 11-14 years	524/999	1.353 (0.802, 2.256)	1.387 (0.817, 2.328)
Age at menarche ≥15 vs 11-14 years	524/999	0.828 (0.562, 1.204)	0.824 (0.557, 1.202)
Nulliparous versus parous [a]	524/999	0.925 (0.656, 1.293)	0.814 (0.573, 1.144)
Parity per 1 child increase [b]	464/877	0.973 (0.897, 1.055)	0.993 (0.915, 1.077)
Age at first birth ≥30 vs <30 years [b]	437/883	1.278 (0.846, 1.915)	1.189 (0.782, 1.791)
Family history of breast cancer yes vs no	524/999	**1.451 (1.132, 1.858)**	**1.465 (1.138, 1.883)**
Previous breast biopsy yes vs no	524/999	**1.815 (1.410, 2.335)**	**1.641 (1.269, 2.122)**
Age at menopause [c]	393/771	**1.126 (1.011, 1.256)**	**1.127 (1.011, 1.259)**
HRT current vs never/former use	524/999	1.054 (0.817, 1.356)	0.931 (0.717, 1.204)
HRT current vs never use	524/999	1.064 (0.790, 1.433)	0.924 (0.681, 1.252)
HRT former vs never use	524/999	1.016 (0.785, 1.316)	0.987 (0.760, 1.284)
HRT current E vs never/former use	524/999	0.934 (0.685, 1.264)	0.838 (0.611, 1.140)
HRT E+P vs never/former use	524/999	**1.932 (1.212, 3.070)**	**1.645 (1.023, 2.636)**
Number of alcoholic drinks per month, per drink increase	524/999	**1.010 (1.000, 1.020)**	1.008 (0.998, 1.018)

Adjusted for age (continuous), state (Minnesota, Wisconsin, Iowa, unspecified), current BMI (continuous), parity/age at first birth (nulliparous, parous age at first birth <30, parous age at first birth ≥30 years), previous biopsy (no, yes, other), and family history of breast cancer (no, yes), any HT use (current, past, never), and alcoholic drinks per month (continuous)

PMD was square-root transformed

Nulliparous versus parous [a]: not adjusted for parity/age at first birth

Parity per 1 child increase [b]: Among parous

Age at first birth ≥ 30 versus < 30 years [b]: Among parous

Age at menopause [c]: categories <30, 30-39, 40-44, 45-49, 50-54, ≥55 years

Table 5.3. Odds ratios (ORs) for breast cancer risk unadjusted and adjusted for PMD in women who were premenopausal at the time of enrollment

Selected risk factor	Cases/controls	OR (95% CI) Unadjusted for PMD	OR (95% CI) Adjusted for PMD
BMI (kg/m^2)	191/356	0.985 (0.958, 1.011)	1.010 (0.978, 1.044)
Height (inch) (adjust for BMI)	191/356	1.015 (0.946, 1.090)	1.013 (0.944, 1.089)
Height (inch) (adjust for weight)	191/356	1.028 (0.956, 1.107)	1.005 (0.933, 1.084)
Weight (lbs) (adjust for height)	191/356	0.998 (0.993, 1.002)	1.002 (0.997, 1.008)
BMI (kg/m^2), current	135/259	0.974 (0.941, 1.006)	1.002 (0.962, 1.043)
BMI (kg/m^2), 5 years ago	135/257	0.973 (0.938, 1.006)	1.000 (0.959, 1.042)
BMI (kg/m^2), 10 years ago	135/256	0.976 (0.936, 1.015)	1.011 (0.963, 1.059)
Age at menarche ≤10 vs 11-14 years	191/356	2.571 (0.908, 7.569)	2.734 (0.950, 8.156)
Age at menarche ≥15 vs 11-14 years	191/356	0.894 (0.471, 1.636)	0.838 (0.440, 1.543)
Nulliparous vs parous [a]	191/356	1.157 (0.813, 1.665)	1.331 (0.928, 1.932)
Parity per 1 child increase [b]	162/307	0.922 (0.762, 1.112)	0.939 (0.774, 1.134)
Age at first birth ≥30 vs <30 years [b]	153/292	1.424 (0.859, 2.345)	1.418 (0.853, 2.341)
Family history of breast cancer yes vs no	191/356	**1.751 (1.097, 2.789)**	**1.787 (1.115, 2.858)**
Previous breast biopsy yes vs no	191/356	1.607 (0.943, 2.727)	1.421 (0.823, 2.437)
Number of alcohol drinks per month, per drink increase	191/356	0.992 (0.972, 1.011)	0.990 (0.970, 1.009)

Adjusted for age (continuous), current BMI (continuous), parity/age at first birth (nulliparous, parous age at first birth <30, parous age at first birth ≥30 years), previous biopsy (no, yes, other), and family history of breast cancer (no, yes), and alcoholic drinks per month (continuous)
PMD was square-root transformed
Nulliparous versus parous [a]: not adjusted for parity/age at first birth
Parity per 1 child increase [b]: Among parous
Age at first birth ≥30 versus <30 years [b]: Among parous

Table 5.4. Total, direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (PMD), among postmenopausal women*

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	**95%CI**	**p-val**	**OR**	**95%CI**	**p-val**	**OR**	**95%CI**	**p-val**	
Without exposure × PMD interaction										
Full dataset†										
BMI (continuous, kg/m^2)	1.06	1.042, 1.093	<.001	0.97	0.956, 0.977	<.001	1.03	1.009, 1.046	**0.004**	<
BMI (continuous, kg/m^2), current	1.06	1.038, 1.084	<.001	0.97	0.956, 0.977	<.001	1.02	1.005, 1.045	**0.013**	<
BMI (continuous, kg/m^2), 5 years ago	1.04	1.014, 1.057	0.001	0.98	0.966, 0.986	<.001	1.01	0.991, 1.030	**0.296**	<
BMI (continuous, kg/m^2), 10 years ago	1.05	1.022, 1.071	<.001	0.97	0.962, 0.983	<.001	1.02	0.996, 1.040	**0.114**	<
Age at menarche ≤10 vs 11-14 years	1.39	0.823, 2.337	0.219	1.01	0.895, 1.135	0.896	1.40	0.819, 2.388	0.220	~
Nulliparous vs parous*	0.81	0.577, 1.151	0.245	1.13	1.040, 1.218	0.003	0.92	0.646, 1.301	0.627	~
First degree family history of BC	1.47	1.140, 1.884	0.003	0.95	0.902, 1.011	0.170	1.40	1.082, 1.810	**0.010**	Not mediated
Breast biopsy/aspiration/lumpectomy	1.64	1.269, 2.120	<.001	1.09	1.025, 1.166	0.007	1.79	1.380, 2.330	**< .001**	18.7%
Age at menopause	1.13	1.012, 1.260	0.030	1.00	0.976, 1.021	0.877	1.13	1.008, 1.260	**0.036**	Not mediated
HRT current vs never/former use	0.93	0.718, 1.206	0.587	1.13	1.056, 1.201	<.001	1.05	0.807, 1.361	0.726	~
HRT current E vs never/former use	0.84	0.614, 1.144	0.265	1.11	1.032, 1.186	0.004	0.83	0.676, 1.270	0.637	~
HRT current E+P vs never/former use	1.65	1.026, 2.637	0.039	1.19	1.048, 1.340	0.007	1.95	1.203, 3.160	**0.007**	32.5%
Alcoholic drinks per month	1.01	0.999, 1.018	0.095	1.00	1.000, 1.004	0.096	1.01	1.000, 1.020	**0.044**	14.97%
Parous women only‡										
Parity (continuous)	0.99	0.915, 1.077	0.864	0.99	0.970, 1.003	0.113	0.98	0.902, 1.064	0.623	~
Age at first live birth 30+ vs <30 years	1.18	0.782, 1.784	0.430	1.09	0.995, 1.199	0.064	1.29	0.846, 1.965	0.237	~
With exposure × PMD interaction										
Full dataset†										

Table 5.4. Total, direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (PMD), among postmenopausal women* (cont.)

	Natural Direct Effect (NDE)			Natural Indirect Effect (NIE)			Total Effect (TE)			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Age at menarche ≤10 vs 11-14 years	1.34	0.776, 2.299	0.297	1.01	0.922, 1.098	0.897	1.34	0.773, 2.335	0.296	~
Nulliparous vs parous*	0.79	0.551, 1.143	0.214	1.18	1.032, 1.343	0.020	0.93	0.641, 1.361	0.724	~
First degree family history of BC	1.54	1.153, 2.068	0.004	0.94	0.863, 1.017	0.120	1.45	1.078, 1.943	**0.014**	Not mediated
Breast biopsy/aspiration/lumpectomy	1.64	1.269, 2.121	<.001	1.09	1.012, 1.178	0.023	1.79	1.375, 2.334	**<.001**	18.7%
Age at menopause	1.13	1.013, 1.263	0.029	1.00	0.981, 1.017	0.877	1.13	1.011, 1.262	**0.031**	Not mediated
HRT current vs never/former use	0.92	0.703, 1.197	0.526	1.15	1.049, 1.251	0.002	1.05	0.805, 1.374	0.714	~
HRT current E vs never/former use	0.83	0.604, 1.138	0.245	1.12	1.017, 1.243	0.022	0.93	0.676, 1.286	0.668	~
HRT current E+P vs never/former use	1.62	0.985, 2.667	0.057	1.20	0.968, 1.498	0.095	1.95	1.199, 3.177	**0.007**	34.3%
Alcoholic drinks per month	1.01	0.999, 1.019	0.086	1.00	1.000, 1.004	0.098	1.01	1.001, 1.021	**0.040**	14.99%
Parous women only‡										
Number of live births (continuous)	1.01	0.920, 1.099	0.906	0.99	0.973, 1.004	0.138	0.99	0.907, 1.088	0.888	~
Age at first live birth 30+ vs <30 years	1.21	0.790, 1.840	0.385	1.05	0.957, 1.163	0.280	1.27	0.835, 1.937	0.262	~

*Postmenopausal women at the time of mammogram. Mediation analysis using the SAS macro by Valeri, L., & VanderWeele, T. J. (2013) and the 95% CIs were estimated using the delta method;
†Multivariate analyses adjusted for age (continuous), current BMI (continuous), parity/age at first birth (nulliparous, parous age at first birth <30, parous age at first birth >=30), previous biopsy (no, yes, unknown), family history of breast cancer (no, yes, unknown), any HT use (current vs past/never), and drinks per month (continuous);
‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (continuous) for parity/age at first birth and further adjusted for age at first live birth (continuous, years);
Nulliparous versus parous *: not adjusted for parity/age at first birth;
§Proportion mediated (PM) was calculated using the following formula if NIE is significant: PM = OR^{NDE} (OR^{NIE} - 1)/(OR^{NDE} × OR^{NIE} - 1); PM calculated to be < 0% and > 100% were denoted by "<" and ">" respectively; It was not calculated (notated using symbol ~) if the total effect is not significant;
**Mediation analysis with exposure-mediator interaction for continuous BMI was not reported due to high multicollinearity detected for the interaction term;
Abbreviations: CI, confidence interval; OR, odds ratio; NDE, natural direct effect; NIE, natural indirect effect; PM, proportion mediated.

Table 5.5. Total, direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (PMD), among premenopausal women*

	Natural Direct (NDE)			Natural Indirect (NIE)			Total			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Without exposure × PMD interaction										
Full dataset†										
BMI (continuous, kg/m^2)**	1.01	0.974, 1.041	0.689	0.98	0.956, 0.995	0.015	0.98	0.955, 1.009	0.192	~
Age at menarche ≤10 vs 11-14 years	2.69	0.932, 7.755	0.067	0.92	0.758, 1.129	0.443	2.49	0.848, 7.287	0.097	~
Nulliparous vs parous	1.05	0.615, 1.809	0.847	1.06	0.967, 1.157	0.224	1.11	0.647, 1.920	0.695	~
First degree family history of BC	1.75	1.090, 2.816	0.021	0.98	0.910, 1.062	0.664	1.72	1.065, 2.784	**0.027**	Not mediated
Breast biopsy/aspiration/lumpectomy	1.49	0.861, 2.568	0.154	1.07	0.966, 1.178	0.199	1.59	0.918, 2.742	0.098	~
Alcoholic drinks per month	0.99	0.968, 1.008	0.233	1.00	0.999, 1.005	0.202	0.99	0.970, 1.010	0.325	~
Parous women only‡										
Parity (continuous)	0.94	0.778, 1.138	0.529	0.97	0.940, 1.007	0.119	0.92	0.756, 1.109	0.366	~
Age at first live birth 30+ vs <30 years	1.44	0.871, 2.380	0.155	1.00	0.929, 1.077	0.998	1.44	0.866, 2.393	0.160	~
With exposure × PMD interaction										
Full dataset†										
Age at menarche ≤10 vs 11-14 years	5.78	0.599, 55.78	0.129	0.73	0.304, 1.738	0.474	4.20	0.509, 34.76	0.183	~
Nulliparous vs parous	1.04	0.603, 1.795	0.886	1.08	0.935, 1.245	0.298	1.12	0.645, 1.954	0.682	~
First degree family history of BC	1.72	1.072, 2.773	0.025	0.99	0.949, 1.037	0.718	1.71	1.061, 2.755	**0.027**	Not mediated
Breast biopsy/aspiration/lumpectomy	1.52	0.862, 2.677	0.148	1.13	0.928, 1.369	0.228	1.71	0.935, 3.136	0.082	~
Alcoholic drinks per month	0.99	0.969, 1.010	0.298	1.00	0.999, 1.006	0.197	0.99	0.971, 1.012	0.418	~
Parous women only‡										
Number of live births (continuous)	0.98	0.795, 1.200	0.822	0.98	0.947, 1.024	0.427	0.96	0.776, 1.191	0.718	~

Table 5.5. Total, direct and indirect effects of exposure on risk of breast cancer, mediated by percent mammographic density (PMD), among premenopausal women* (cont.)

	Natural Direct (NDE)			Natural Indirect (NIE)			Total			Proportion Mediated§
	OR	95%CI	p-val	OR	95%CI	p-val	OR	95%CI	p-val	
Age at first live birth (continuous)	1.44	0.867, 2.380	0.160	1.00	0.924, 1.082	0.998	1.44	0.862, 2.395	0.165	~

*Premenopausal women at enrollment. Mediation analysis using the SAS macro by Valeri, L., & VanderWeele, T. J. (2013) and the 95% CIs were estimated using the delta method;
†Multivariate analyses adjusted for age (continuous), current BMI (continuous), parity/age at first birth (nulliparous, parous age at first birth <30, parous age at first birth >=30), previous biopsy (no, yes, unknown), family history of breast cancer (no, yes, unknown), and drinks per month (continuous);
‡Multivariate analyses restricted to parous women. In addition to risk factors listed in footnote †, replaced number of live births (continuous) for parity/age at first birth and further adjusted for age at first live birth (continuous, years);
Nulliparous versus parous *: not adjusted for parity/age at first birth;
§Proportion mediated (PM) was calculated using the following formula if NIE is significant: PM = OR^{NDE} (OR^{NIE} - 1)/(OR^{NDE} × OR^{NIE} - 1); PM calculated to be < 0% and > 100% were denoted by "<" and ">" respectively; It was not calculated (notated using symbol ~) if the Total effect is not significant;
**Mediation analysis with exposure-mediator interaction for continuous BMI was not reported due to high multicollinearity detected for the interaction term;
Abbreviations: CI, confidence interval; OR, odds ratio; NDE, natural direct effect; NIE, natural indirect effect; PM, proportion mediated.

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CHAPTER 6: SUMMARY AND CONCLUSIONS

6.1 SUMMARY

6.1.1 Comparison of Study Findings

This section aims to compare the results from the Seattle study and the Mayo study. While the subjects in the Seattle study were mainly premenopausal women, those in the Mayo studies were mostly postmenopausal. Although the characteristics of the subjects differ, both studies found similar conclusions regarding mediation for some of the breast cancer risk factors. This comparison is summarized in Table 6.1.

The first breast cancer risk factor with consistent findings regarding mediation is a first-degree family history of breast cancer. Both studies consistently found that the increased risk of breast cancer due to a history of breast cancer in the first-degree family member was not mediated by mammographic density, regardless of pre- or postmenopausal status. This indicates that a first-degree family history of breast cancer may increase breast cancer risk through pathways independent of mammographic density. Therefore, for women who have an increased risk of breast cancer because of a first-degree family history of breast cancer, having a lower breast density would not decrease their breast cancer risk.

The second breast cancer risk factor with a consistent finding regarding mediation is a history of breast biopsy. Both studies showed that a portion (18.7-83.1%) of the excess risk in relation to a history of breast biopsy was mediated by percent mammographic density. In the Seattle study, partial mediation was found for the increased risk associated with prior identification of breast calcifications. This suggests that breast lesions such as a history of breast biopsy and calcifications on mammograms may increase breast cancer risk through increasing breast density. However, the results must be interpreted with caution given that it is unknown whether breast lesions occur after an increase in breast density. We cannot rule out the possibility that it may occur before or just at the same time as the breast density changes.

The third breast cancer risk factor with a consistent finding regarding mediation is BMI. Both studies observed inconsistent mediation or suppression for adult BMI. Suppression occurs

when there are multiple pathways with opposing directions between a risk factor and an outcome. On the one hand, increasing BMI has been found to be negatively associated with percent mammographic density [1-3], which in turn would reduce breast cancer risk. On the other hand, increasing BMI appeared to increase breast cancer risk through pathways independent of percent mammographic density. The overall total effect depends on these two opposing effects, which may seem to disappear when these two effects cancel out each other. This may be the reason why the total effects for BMI among pre-menopausal women were found to be not significant. However, the total effect for current BMI among postmenopausal women was found to be positive and significant. We observed that the direct effect among postmenopausal women independent of mammographic density appeared to be stronger than the direct effect via mammographic density. This observation supports the hypothesis that the increased risk of breast cancer associated with greater BMI in postmenopausal women is largely mediated through pathways independent of mammographic density such as those involving estrogen levels. This can also explain why BMI appeared to affect the risk of breast cancer for pre- and postmenopausal women differentially.

Among the reproductive risk factors, nulliparity is the one most likely to be mediated by mammographic density. Although the total effect was not significant in the Mayo study, the NIE was found to be significant in both studies, indicating a potential mediation. As for postmenopausal hormone replacement therapy, current use of combined estrogen and progestin was found to be 32.5% mediated by percent mammographic density in the Mayo data. However, this factor was not considered in the Seattle data set because women in the Seattle study had their mammograms before age 50 years old and less than 10% of them used hormone replacement therapy, a majority of whom took the hormone replacement therapy after their mammographic density measures.

6.1.2 Comparison of Study Findings to the Literature

This section aims to compare the results based on the Seattle and Mayo datasets to the findings from the literature. Despite heterogeneous participant characteristics among studies, some of the findings regarding mediation were found to be consistent with other studies. The comparison is summarized in Table 6.2.

The findings from the Seattle and Mayo studies are in general consistent with the literature for some of the risk factors (Table 6.2). Percent mammographic density was found to partially mediate the associations between a history of a breast biopsy and current use of combined estrogen and progesterone hormone replacement therapy with risk of breast cancer, suggesting that these risk factors at least partially influence breast cancer risk through changes in breast tissue density. On the other hand, all studies consistently showed that percent mammographic density did not mediate the associations between family history of breast cancer and breast cancer risk in both pre- and postmenopausal women. Furthermore, inconsistent mediation or suppression was observed for adult BMI across all studies. For other risk factors, further studies are needed to determine the mediation effect of mammographic density given inconsistent results.

6.2 CONCLUSIONS

In conclusion, percent mammographic density partially mediated the associations between prior breast biopsy and breast cancer risk in both pre- and postmenopausal women as well as mediated the association with combined hormone replacement therapy use of estrogen and progesterone among postmenopausal women. However, the mediation results for prior breast biopsy must be interpreted with caution given that a causal relationship has not been established between breast lesions (reflected by a breast biopsy) and an increase in breast density (detected in a mammogram). We found that only 10-33% of the association between current use of combined HRT and breast cancer risk was mediated by percent mammographic density. While there might be a potential mediation for the association between ever parous and current use of HRT overall with the risk of breast cancer, further studies are needed to confirm the mediation effect. A suppression effect or inconsistent mediation was observed for adult BMI. For the rest of the risk factors, there appeared to be not much mediation. Particularly, the increased risk in relation to a family history of breast cancer and older age at menopause was found to be not mediated by percent mammographic density. This work suggests that the utility of percent mammographic as an intermediate marker of breast cancer risk may be limited. Additional research is necessary to confirm these observations as well as to explore the extent to which mammographic density mediates the associations with other established breast cancer risk factors.

TABLES

Table 6.1. Comparison of causal mediation analysis results between the Seattle Data and the Mayo Data

Risk Factor	Seattle Study (Case-control)			Mayo Study (Nested case-control)			Conclusions
	Mostly Premenopausal			Mostly Postmenopausal			
	TE	NIE	PM	TE	NIE	PM	
1st Degree family history of BC	sig*	ns	Not mediated	sig*	ns	Not mediated	Not mediated
Previous breast biopsy/lumpectomy	sig	sig*	83.1%	sig*	sig*	18.7%	Partially mediated
Breast calcifications classification	sig*	sig*	20.5%	~	~	~	Partially mediated
Parous vs nulliparous	sig*	sig*	48.6%	ns	sig*	Not calculated	Partially mediated
Parity per 1 child increase (parous only)	ns	sig*	Not calculated	ns	ns	Not calculated	Not determined
Age at 1st live birth (parous only)	sig*	ns	Not mediated	ns	sig	Not calculated	Not mediated (premenopausal women)
Age at menopause	~	~	~	sig*	ns	Not mediated	Not mediated
HRT, current vs never/former	~	~	~	ns	sig*	Not calculated	Not determined
HRT (E), current vs never/former	~	~	~	ns	sig*	Not calculated	Not determined
HRT (E+P), current vs never/former	~	~	~	sig*	sig*	32.5%	Partially mediated
Smoking ever vs never	sig	ns	Not mediated	~	~	~	Not mediated
Alcoholic drinks per month	~	~	~	sig*	sig	14.97%	Partially mediated
BMI (kg/m^2), adult	ns	sig*	Inconsistent mediation (NIE-, NDE+)	~	~	~	Inconsistent mediation
BMI (kg/m^2), age 18 years	ns	sig*	Inconsistent mediation (NIE-, NDE+)	~	~	~	
BMI (kg/m^2), current	~	~	~	sig* (+)	sig*	Inconsistent mediation (NIE-, NDE+)	
BMI (kg/m^2), 5 years ago	~	~	~	ns	sig*	Inconsistent mediation	

(cont.)

Table 6.1. Comparison of causal mediation analysis results between the Seattle Data and the Mayo Data

Risk Factor	Seattle Study (Case-control)			Mayo Study (Nested case-control)			Conclusions
	Mostly Premenopausal			Mostly Postmenopausal			
	TE	NIE	PM	TE	NIE	PM	
						(NIE-, NDE+)	
BMI (kg/m^2), 10 years ago	~	~	~	ns	sig*	Inconsistent mediation (NIE-, NDE+)	

Abbreviations: BC: breast cancer; E: estrogen; E+P: estrogen plus progesterone; BBD: Benign breast disease; TE: total effect; NIE: natural indirect effect; NDE: natural direct effect; PM: proportion mediated; sig: significant (p-value <0.1, p-value <.05 if followed by an asterisk (*)), ns: not significant

Table 6.2. Comparison of our causal mediation analysis results to other studies

Risk Factor	**Seattle & Mayo study results**	**Literature Review (Chapter 2)**	**Rice M. *et al.* 2016, 2018 [4, 5]**		**Summary**
			Premenopausal	**Postmenopausal**	
1st Degree family history of BC	Not mediated	Small PE (≤14%)	Not mediated	Not mediated	Not mediated
Prior breast biopsy/BBD	Partially mediated (PM = 18.7-83.1%)	PE = 12-73%	PE = 17%	PE = 24%-33%	Partially Mediated
Ever parous	Partially mediated (PM = 48.6%, premenopausal)	PE = 14-52%	TE (ns), PE = 40-52% (ns)	Either not mediated or PE = 43-52%	Further studies are needed
Parity per child increase (parous only)	Not determined	Inconclusive	Not mediated	Not mediated	Further studies are needed
Age at 1st live birth (parous only)	Not mediated (premenopausal women)	PE = 16-17%	Not mediated	PE = 13-16%	Not mediated for premenopausal women
Age at menopause	Not mediated	~	~	Not mediated	Likely not mediated
HRT, current vs never/former	Not determined	PE = 10-37%	~	PE = 22-37%	Further studies are needed
HRT (E), current vs never/former	Not determined	Inconclusive	~	PE = 69%	Further studies are needed
HRT (E+P), current vs never/former	Partially mediated (PM = 32.5%)	PE = 10-26%	~	PE = 26%	Partially mediated

Table 6.2. Comparison of our causal mediation analysis results to other studies (cont.)

Risk Factor	**Seattle & Mayo study results**	**Literature Review (Chapter 2)**	**Rice M. *et al.* 2016, 2018 [4, 5]**		**Summary**
			Premenopausal	**Postmenopausal**	
Smoking ever vs never	Not mediated	~	~	~	Further studies are needed
Alcoholic drinks per month	Partially mediated (PM = 14.97%)	~	Not mediated	PE = 16-73% (ns)	Further studies are needed
Adult BMI	Inconsistent Mediation	All |PE| > 100%	TE (ns), IE (-) DE (+)	TE (+), IE (-) DE (+)	Inconsistent Mediation

Abbreviations: BC: breast cancer; E: estrogen; E+P: estrogen plus progesterone; BBD: Benign breast disease; TE: total effect; IE: indirect effect; DE: direct effect; PM: proportion mediated; PE: proportion explained; sig: significant; ns: not significant

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